Studies on the Effect of Insecticide Combinations on Culex Mosquito Larvae

A Thesis Submitted
In Partial Fulfilment of the Requirements
for the Degree of
MASTER OF TECHNOLOGY

By

M. P. Pandey

to the
DEPARTMENT OF CIVIL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY KANPUR

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To
My Mother
affectionately dedicated

CERTIFICATE

This is to certify that the present work has been done by Shri M.P. Pandey under my supervision and the work has not been submitted elsewhere for a degree.

Dr. A.V.S. Prabhakara Rao
Associate Professor
Department of Civil Engineering
Indian Institute of Technology Kanpur

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Experimental investigations on the effect of combinations of insecticides as larvicides are presented in this study. The insecticides used include DDT, Endrin, Aldrin the chlorinated hydrocarbon, Thimate an organophosphorus insecticide, pyrethrum extract, a plant derivative and piperonyl butoxide, widely used synergist. A special emphasis is placed on evaluating the best combinations exhibiting synergism. Relative toxicities of the insecticides, acting alone and in combinations are compared and discussed.

1. INTRODUCTION

It is estimated that filariasis, in India, is responsible for a national loss of about Rs. 100 crores per year. About 25 million people in India were estimated to be living in filarious areas during 1953 (1). The forth plan envisages protective measures to over 12 million people in the urbon area. The etological agents of filariasis are W. bancrofti and B. malayi, which are commonly known as filarial worms. The microfilariae that are in the blood of infected persons are picked up by mosquitoes, Culex - fatigans. One of the major methods of controlling vector born diseases in envoirnmental sanitation is to eliminate the vectors.

Many of the mosquitoes belonging to the genus culex, generally called culicines, are disease vectors while others require control because of the annoyance and discomfort they cause. Extensive larvicidal treatments with single synthetic insecticide often brought about the development of resistance (2). Culex - fatigans, the major transmitter of filariasis is generally not as susceptible to DDT or other chlorinated insecticides as other insects. The dosages of these insecticides may have to be increased two to three times that is usually employed for satisfactory control of other mosquitoes (1).

The WHO expert committee on insecticides (1963) considering the lines of future research emphatically recommended that information should be obtained on conditions in which DDT may still be used effectively against DDT resistant population (2)

1.1 Importance of insecticides used in combinations

Inspite the large number of synthetic insecticides, pyrethrum extract is still one of the best natural insecticides. However being a natural product it is expensive and not easily available. Pyrethrum extract is a mixture of pyrethrins and cinerins which are found in the flowers of Chrysanthemum cinereriaefolium and Chrysanthemum coccineum (3). For a variety of reasons, the commercially available insecticidal formulations have more than one insecticide. Each compound contributes to the mixture, a desirable specific property. This is evident in the mixture of pyrethrum and DDT which contribute to a quick paralytic action and strong lethal effect respectively (4).

A mixture is useful in controlling a mixed population of insects because of one specie is very susceptible to one component, the other specie may be susceptible to second component.

Indiscriminate wide use of certain insecticide has resulted in the development of resistance in insects to that insecticide. Hewlett and Placket (5) have proposed the use of mixtures of insecticides of independent action as a method that is less likely to induce resistance to individual poisions, in insects.

Exploiting the synergistic property by arriving at a mixture of compounds may permit more economical control of insects rather than by an individual insecticide alone.

Mixture of two or more compounds may reduce toxicity to higher animals with no reduction in toxicity to insects. For example ethyl-parathion has been marketed as a mixed formulation with methyl-parathion for reducing the toxicity to higher animals (6).

An investigation into the joint action of insecticides is, therefore felt to be, extremely important from the point of effective insect control and to prevent the development of resistance in insects.

Numerous insecticidal formulations containing more than one active ingradient are at present used in practice in our country viz. Flit, Endrix - M, New-spray, Shell-Tox etc. However no systamatic work seems to have been attempeted on the effect of combination of chemicals with reference to larvicides. Selection of the combinations appear to be purely emperical. A methodical investigation, using some of the popular insecticides in conbinations may yield valuable information in the control of Culex mosquitoes that are responsible for transmitting various contagious diseases.

1.2 Aim

To determine the relative toxicity of individual insecticide to culicine larvae.

To evaluate the concentrations of insecticides in combination that gives higher mortalities of culicine larvae.

To compare various combinations of insecticides with respect to joint action yielding higher percentage of mortality of above larvae.

1.3 Scope

In this study the work is centred around in finding a combination of commonly used insecticides yielding high mortality of culex mosquito larvae. Inseticides used in this study include chlorinated hydrocarbons, organophosphorus compound, plant insecticide and an activator.

2. LITERATURE REVIEW

The use of pesticides in public health programmes and agriculture dates back to nearly 200 years. Insecticides from plant products were introduced in United States in the year 1858 (7). A revolutionary change was brought in the control of communicable diseases with the introduction of organic insecticides like DDT and Dieldrin around 1930 (7).

2.1 Principle types of insecticides

Chlorinated hydrocarbon insecticides are very popular in India because of their availability and low cost. There were nearly 500 insecticides available in more than 54000 formulations in United States alone by 1962 (8). Some of the common chlorinated hydrocarbon insecticides are, DDT, BHC, Chlordane, Endrin, Aldrin etc.

Due to indiscriminate use of chlorinated hydrocarbon insecticides, specially DDT, the insects developed resistance to them (2). Organo phosphorus insecticides of proven effectiveness substituted the chlorinated hydrocarbon insecticides. Some of the organophosphorus insecticides are, Diazion, Malathion, Thimate, Sumathion etc.

Insecticides of curbamite group are developed recently and they are only in experimental stage in India. Sevin, an Union Carbide (9) product has gained popularity as it is very effective in controlling the agricultural pests. Other insecticides. from this group are, Pyrolon, Ferbam, Chloro IPC etc.

Pyrethrum, a plant product, was introduced in United States in the year 1858 (7). Other plant product used as insecticide is the rotenone. Out of these two, pyrethrum is very much popular due to its quick paralytic action (4). Being a natural product, it is expensive and may not be easily available.

2.2 Economic importance

Annual loss due to prevalance of disease, filariasis alone, is estimated to be Rs. 100 crores in India (1). Before the introduction of these potential insecticides in public health programmes every year 100 million people suffered from malaria, out of which one million people died costing the national loss about Rs. 1000 crores annually (10). The proportionate case rate of malaria came down from 10.8% in 1953-54 to 0.04% in 1967, showing a reduction of 99.7%. The annual morbidity of 75 millions in 1952-53 is reduced to 0.28 million in 1967-68 due to intensive insect control programme.

2.3 Development of resistance

Definition of resistance as given by WHO expert committee on the insecticides is "Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which will prove lethal to the majority of individuals in a normal population of the same species" (11).

Barely a decade after the introduction of the potent synthetic insecticides in public health programmes, the main

technical problem is the development of resistance to them by the insects they formerly controlled. The first case of DDT resistance to housefly and culex mosquitoes was reported in Italy in 1947 (12). The WHO expert committee on insecticides in 1956 reported that there was clear evidance of resistance in 20 insect species of public health importance (13). In 1962, the number of species of public health importance for which there was sure evidance of resistance had increased to 81 species (14).

The development of resistance in insects of the field is mainly to chlorinated hydrocarbons. If all the instances of resistance, including agricultural insects, are considered, it is found that over half involve resistance to DDT, and as much as five-sixths to some chlorinated hydrocarbon (11). There are two reasons given for this. Firstly, the insects showed varying degrees of reaction to the chlorinated hydrocarbons and hence a wide range of dosage levels would exert on the pupulation and make the insecticides 'resistance - prone'. The other factor which helps the development of resistance to these insecticides is their long residual activity. For this reason, Henderson (15) suggested that the insecticide choosen for malaria control should be one whose residue deteriorates rapidly at the end of transmission season.

2.4 Cross-resistance

In the literature it is indicated that the houseflies which have developed resistance to one of the chlorinated

hydrocarbon insecticides, such as DDT need not necessarily show resistance to other compounds from the same group like B.H.C., dieldrin, and heptachlor. Brown reports that in general it is a rule that DDT resistant strains show no significant cross-resistance to gamma-BHC (11). In a review published in 1955, Kearns (16) stated that the 40 different resistant strains examined in his laboratory all fell into one of the following classes.

1. Resistant to DDT and analogues, susceptible to gamma-BHC, dieldrin and analogues, developed by field laboratory pressure from DDT.

2. Resistant to gamma-BHC, dieldrin and analogues, susceptible to DDT and analogues, developed by laboratory pressure from gamma-BHC or dieldrin.

Chlorinated hydrocarbon compounds may not induce a cross-resistance to organophosphorus compounds. The DDT-resistant house-fly strain was found by Barber and Schmitt to show a normal susceptibility to parathion (17).

The strong and specific resistance to chlorinated hydrocarbon need not involve any cross-tolerance to pyrethrins at all as examplified by resistant field strains from England, Sardinia, Newyork and California (11). Multiresistant laboratory strains either showed no pyrethrin tolerance or atmost an increase in dose which does not exceed twice the normal even in the most highly resistant strain. One of the reasons for the slow development of resistance to pyrethrum is attributed to the lack

of its residual action. In a few experimental cases where resistance was induced specifically to pyrethrum showed that those insects were resistant to some of the synthetic insecticides also (18).

2.5 Joint action of insecticides

The study of the toxic action of compounds involves the knowledge of their behaviour when two or more applied jointly. In some cases, potency of a mixture may be greater than would be expected simply from a knowledge of the potencies of the individual compounds. This is of importance in the economic utilization of poisions. Cases of reduced potency of a mixture by comparision with that of its constituents may also occur.

The first systematic study of this topic was given by Bliss (19). His concept has been extended by Finney (20). Finney distinguished four types of joint actions, i.e. independent, similar, synergistic and antagonistic.

Independent action:

The insecticides act independently and have different modes of toxic action. The susceptibility of one component may or may not be correlated with the susceptibility to the other. They may act seperately on different physiological systems. One constituent may not affect either the amount of the other or reaction of the other.

Similar action;

When th constituents applied jointly produce a common

response as produced individually for example attacking the same physiological system, their action is said to be similar. In this type of action one component can be substituted at a constant proportion for the other.

Synergistic action:

When the insecticides applied jointly produce a total response greater than the sum of their individual effects, the action is known as synergistic and this phenomenon is called 'Synergism'. A special case of synergism may arrive when a substance with no toxicity at the dose employed, increases the effect of other toxicant.

Antagonistic action:

This is just reverse of synergism. In this the total effect of the mixed constituents will be less than the seperate effect of the most toxic constituent alone.

2.6 Joint action of insecticides of plant origin mixed with insecticides of other groups.

While enough work has been done on the effect of mixed insecticides on agricultural pests, very little literature is available on the insects of public health importance.

Beroza (21) studied the effect of pyrethrum mixed with methylenedioxyphenoxy against house-flies and found that strong synergism exhisted, Roberts (22) reported that when pyrethrum mixed with piperonyl-butoxide, showed synergistic effect against house-flies but the mixture failed to increase the mortality of

stable-flies. In the formulation of 'Flit' an Esso product, pyrethrum has been used as synergist in combination with DDT and BHC, for controlling the mosquitoes and housefiles.

Many compounds like phlthalets, N-substituted-benzamides, N-substituted p-bromobenzene sulfonamides and p-toulene sulfonamides are also recommended as synergists (3). MGK 264* is an other synergist commonly used in practice. Methylenedioxyphenyl compounds commonly used as pyrethrum synergist, appear to be generally superior to synergist MGK 264 against housefiles (3).

Pyrethrum has been used in combination with several insecticides and activators in pest control of agricultural significance. Strong (23) reported that the addition of 0.5% rotenone, a natural product, with 0.225% pyrethrum increased the toxicity of pyrethrum considerably against agricultural pests. Also rotenone with sulfur proved to be highly toxic to Burchus brachialis, an agricultural pest, in comparision to rotenone alone, according to the report of Anand (24). Tests conducted at Kansas by Wilber and Donalds (25) showed that pyrethrum mixed with piperonyl butoxide applied in recommended dosages in approved manner prevented damage to stored wheat and barley.

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* Registered trade mark of McLaughtin Gormeley King Company.

2.7 Joint action of chlorinated hydrocarbons with compounds of organophosphorus group

Two phosphorus compounds, gusathion and dipterix and several chlorinated hydrocarbons were tested singly and in combinations against cottom pests by Kamel etal.(26). They concluded that gusathion mixed with DDT proved to be the best combination.

Parencia etal.(27) reported that the mixture of toxaphene, DDT and guthion gave better control of boll-weevil, Anthononus grendis, than dieldrin and toxphene alone. Elemer and Carson (28) used DDT, ethion-dylox (dimethyl - 2,2,2- trichloro-1-hydroxyethyl phosphate), thiodon, thrithion and disyston alone and in combination against lygusbug, Lygus hesperus knight in California. All applications containing thiodon and another containing DDT and dylox were considerably toxic.

According to a report from Veliscol Carporation U.S.A. (29) endrin combined with methyl-parathion controlled all the insects damaging stored grains. Methyl-parathion provided quick knock-down to complement endrin's lasting action. Lakhe (30) worked on the resistant strain of German-cockroaches and found malathion mixed with dieldrin to be most effective.

2.8 Joint action among chlorinated hydrocarbon insecticides

Turner (31) used the following combinations of insecticides against milkweed-bug, Oncopeltus fasciatus.

(i) Chlorodane with — DDT, Methoxychlor, perthane and aldrin.

(ii) Aldrin with — DDT and perthane.

(iii) Dieldrin with — DDT and perthane.

(iv) Lindane combined with — DDT, methoxychlor, perthane and dieldrin.

Most of these combinations showed similar action exacepting dieldrin with DDT and perthane produced diversing mortality curves which might be accepted as due to the interaction.

Brazzel and Lindquist (32) studied the action of insecticides on susceptible and resistant strains of boll-weevil. Toxaphene and DDT exhibited synergistic effect on resistant strain while only additive effect on susceptible strain.

2.9 Joint action of insecticides of carbamate group with insecticides of other groups.

Chapman (33) conducted insecticidal tests against DDT resistant pink bollworm at Texas. Carbryl and a mixture of guthion and DDT gave excellent control of the insect. Georghion (34) used sevin with piperonyl-butoxide against resistant strain of house-flies and concluded that carbamate resistance was in part due to a compound insensative to the action of piperonyl-butoxide. Tahori (35) showed that house-flies, of Israel resistant to DDT were susceptible to sevin and piperonylbutoxide mixture.

2.10 Miscellaneous cases of synergism with activators.

Spiller (36) studied the joint action of DDT and N-N-dibutyl-p-chloro-benzene-sulfonamide and concluded that the mixture was much effective than DDT alone against DDT resistant house-flies, Hoffman (37) exposed DDT resistant house-flies to the mixtures of methylenedioxyphenyl compounds and esters of succinamic and glutamic acids with malathion, methylparathion, diazinon and other phosphorus insecticides. Many of these combination showed synergism.

3. MATERIALS, METHODS AND EXPERIMENTS

Among the various insects of public health importance, mosquitoes have been choosen for the present work because of the wide variety of diseases transmitted by them. The life cycle of mosquito is briefly discussed below.

3.1 Life cycle of mosquito:

The general classification of mosquitoes is as follows(38):

Order	Diptera
Family	Culicidae
Sub-family	Anophelinae
Genus	Anopheles
Sub-family	Culicinae
Genus	Ades, culex etc.

There are four well defined stages in the life history of mosquitoes, viz., the egg, larva, pupa and adult. The first three stages occur in water and the adult is an active flying insect feeding on plant juices and blood from worm-blooded-animals.

Although a female mosquito can live on plant juices, it requires blood meal before laying the eggs. Blood meal is needed after mating. Shallow stagnant water ponds are preferred for laying eggs. The eggs are laid in the form of a raft. About 200 to 300 eggs are laid in a raft by Culex and Ades. Anopheline eggs are laid singly. The time required for the hatching of the eggs is normally two to three days.

Hatching of eggs produce the larvae that live in polluted waters. Larvae subsist on organic matter and bacteria. They have to come to the surface for respiration from time to time. The larvae period includes four developmental stages or 'instars' and at the end of each instar the larva sheds its skin or molt by means of which the instar of larva can be easily detected. Only after the fourth instar completion pupa appears. This takes about 7 to 10 days in total after hatching. The characterstic position of larvae in water makes possible to identify them. While anopheline larvae lie parallal to the surface, most of the other groups hang head down with air vent tube penetrating the surface film.

The mosquito pupa also lives in water. Pupa differs in shape and appearance from larva and does not need any food, but respirs through trumpets. This stage losts normally for 24 hours. After this the skin is broken and the adult mosquito emerges out. The adult mosquito is a small fragile insect with a slender abdomin, one pair of narrow wings and three pairs of long slender legs. The total time from laying of eggs to the flying adult is from 10 to 12 days. Usually the life of an adult mosquito is about 60 days. Peak of culex breeding is in spring. It likes moderate temprature and 80% humidity.

3.2 Selection of Insecticides.

In this investigation the insecticides, DDT, Endrin and Aldrin belong to the chlorinated hydrocarbon group, Thimate

from organophosphorus group while Pyrethrum is a plant product. Piperonyl-butoxide is a synthesized compound used as an activator. Chlorinated hydrocarbon insecticides are used widely in India while organophosphorus have been introduced recently. Piperonyl-butoxide has been selected due to its synergistic property with certain insecticides as pyrethrum.

3.2.1 <u>Chlorinated hydrocarbon insecticides</u>.

DDT or pp'-dichlorodiphenyl-trichloroethane has the chemical name, 2,2 bis-(p-chlorophenyl) 1,1,1-trichloroethane, with the structural formula as shown in fig. no. 1.

DDT has a molecular weight of 345.5. DDT is a white crystalline solid, but produced technically as a white amorphous powder. It has specific gravity equal to 1.556 at 25°C. DDT is almost insoluble in water. DDT is freely soluble in most organic solvants. Because of its very low volatility it has an outstanding insecticidal activity (39).

DDT is probably the most widely used insecticide now available. It is used for variety of purposes in agriculture, in the control of insects of public health importance and in various household uses. Because of its very wide use DDT is more likely to be encountered than any other single insecticide.

DDT is absorbed from the intestinal tract and if it occors in the form of a very fine aerosol or dust it may be taken into the alaveoli of the lung from which it is absorbed readily. DDT is not, however, absorbed through the skin unless it is in solution. Solutions are absorbed through the skin and, by the same

token, emulsions are absorbed to some extent (4).

Endrin also a chlorinated hydrocarbon has the chemical name, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-5,8-dimethanonapthalene (39). Its structural formula is shown in fig. no. 1.

Endrin is available as dusts, granules water weltable powders, and emulsifiable concentrates. Endrin is used for controlling insects of agriculture and public health importance. It is absorbed readily through skin as well as through respiratory and gastrointestinal systems. Like many other chlorinated hydrocarbons acts as a stimulant to the central nerve system (4,7).

Aldrin, an other chlorinated hydrocarbon has the chemical name, 1,2,3,4,10,10-hexchloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene. Its structural formula is shown in fig. no. 1 (38).

Aldrin is a white crystalline solid. It is very soluble in organic solvents and is soluble in water also. It is stable in the presence of most insecticides. It's used in pest control of agricultural importance. Its insecticidal action is similar to other insecticides of this group.

3.2.2 Organophosphorus insecticide.

Thimate is from organophosphorus group. Its chemical name is O,O-Diethyl S-(ethyl thiomethyl)-phosphorodithioate. Its structural formula is shown in fig. No. 1.

Technical thimate is a clear liquid with low water solubility. It is highly soluble in xylene, oils, alcohols,

ethers etc. It is available in grannular form also. Thimate is poisionus by skin contact, inhalation or swallowing. It is highly toxic to agricultural pests (40).

3.2.3 Natural insecticide.

Pyrethrum is obtained from pyrethrum flowers. It contains pyrethrin I and pyrethrin II. The structural formula is shown in fig. no. 2.

Pyrethrum is available commercially in powder or dust form, in a variety of solvent extracts. Pyrethrum alone or combined with synergists is used extensively against a wide variety of insects. It may be absorbed from the gastrointistinal tract and by the respiratory route. It's not obsorbed to a significant degree through the skin (4,39,41).

3.2.4 Synthetic activator.

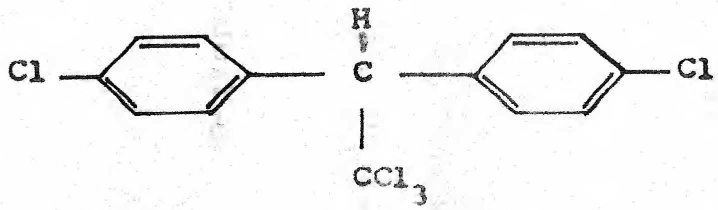
Piperonyl butoxide is a synthesized compound having chemical name, (3,4-methylenedioxy-6-propyl benzyl)(butyl) diethylene-glycolether. Its structural formula is shown in fig. no. 2.

Piperonyl butoxide in the technical grade, is nearly odorless, paleyellow, oily liquid, having specific gravity of about 1.06. It is soluble in all dilutions of mineral oils. It is mostly used as an activator with pyrethrum against mosquitoes and houseflies (7).

3.3 Selection of solvents.

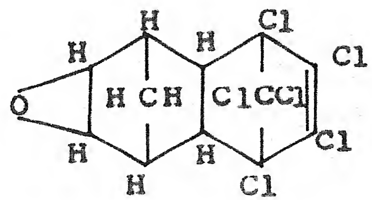
The aqueous solubility of the insecticide is important as the larvae are acquatic. Since the aqueous solubility of these

DDT



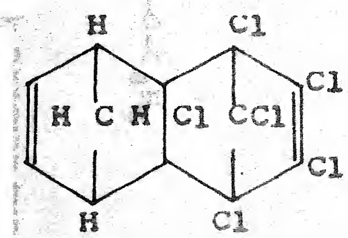
1,1,1-TRICHLORO-2,2-DI(p-CHLOROPHENYL)-ETHANE

ENDRIN



1,2,3,4,10,10-HEXACHLORO-6,7-EPOXY-1,4,4a,5,6,7,8,8a-OCTAHYDRO-1,4-ENDO-ENDO-5,8-DIMETHANONAPHTHALENE

ALDRIN



1,2,3,4,10,10-HEXACHLORO-1,4,4a,5,8,8a-HEXAHYDRO-1,4,5,8-DIMETHANONAPHTHALENE

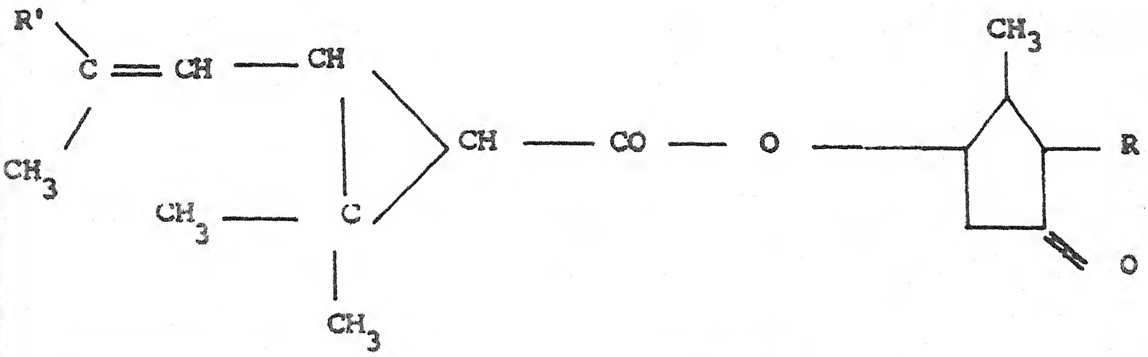
THIMATE

$$(C_2H_5{-}O)_2{-}\overset{\overset{\displaystyle S}{\|}}{P}{-}S{-}CH_2{-}S{-}C_2H_5$$

O,O-DIETHYL S-(ETHYLTHIOMETHYL) PHOSPHORODITHIOATE

FIG. 1 CHEMICAL FORMULAE OF INSECTICIDES

PYRETHRUM



PYRETHRINE I

R = $- CH_2-CH=CH-CH=CH_2$

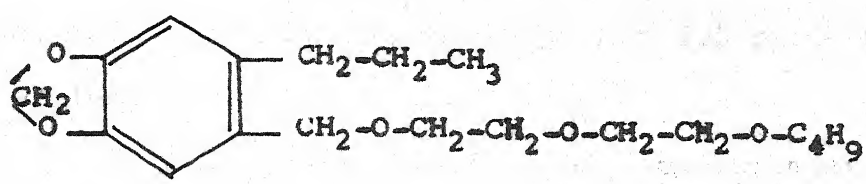
R' = $- CH_3$

PYRETHRINE II

R = $- CH_2-CH= CH-CH=CH_2$

R' = $- CO-O-CH_3$

PIPERONYL BUTOXIDE



3,4-METHYLENEDIOXY-6-(PROPYL BENZYL)(BUTYL) DIETHYLENE-GLYCOL ETHER

FIG. 2 CHEMICAL FORMULAE OF INSECTICIDES

compounds is less, another solvent namely ethanol was used. A limitation of solvent itself in this type of study is the toxicity of the solvent itself to the insect. However a control with solvent was used in all experiments to eliminate any error.

3.4 Experiments.

Larvae were collected from the ponds of near-by villege. The larvae choosen were in their late third instar or earlier fourth instar. Any larva showing abnormalities was discarded. Twenty larvae were isolated by means of small strainer into the beakers containing 24 ml. of water.

The required number of 500 ml.beakers were filled with 225 ml.of water. The test concentrations were prepared by pipetting one ml. of the appropriate standard insecticidal solution under the surface of the water in each of the beakers and then mixed by stirring. After 15 minutes of mixing the test concentrations the mosquito larvae were transferred from the small small beakers with 24 ml. of water, thus bringing the total volume in each beaker to 250 ml. The maximum deapth of water was more than 2.5 cms. and less than 7.5 cms. which is optimum for larvae (2).

In case of the mixture of two or more insecticides, the solutions of same strength of different insecticides were prepared and mixed to give the required proportion on volumetric basis. From the mixture thus obtained, test concentrations were prepared. A control with each set was also kept.

The mortality counts were made after 24 hours of exposure time to the insecticide. When ever 10% or more larvae pupated out in the course of experiment in any beaker the test was discarded. The test was repeated when ever the control mortality was 20% or more. When the control mortality was less than 20% a correction has been applied in the data analysis.

The insecticides were used jointly in the following proportions.

	DDT	DDT	DDT
Endrin	1:1	1:9	1:19
Aldrin	1:1	1:4	1: 9
Thimate	1:1	1:9	1:19
Pyrethrum	1:1	1:9	1:19
Piperonyl butoxide	1:1	2:1	
	Endrin	Endrin	Endrin
Aldrin	1:1	2:1	
Thimate	1:1	2:1	1:2
Pyrethrum	1:1	2:1	1:2
Piperonyl butoxide	1:1	2:1	4:1
	Aldrin	Aldrin	Aldrin
Thimate	1:1	1:2	
Pyrethrum	1:1	1:2	
Piperonyl butoxide	1:1	2:1	4:1

	Thimate	Thimate	Thimate
Pyrethrum	1:1	2:1	1:2
Piperonyl butoxide	1:1	2:1	4:1
	Pyrethrum	Pyrethrum	Pyrethrum
Piperonyl butoxide	1:1	2:1	4:1

Mortality counts were taken after 24 hours. The insects were grouped into the following categories for recording the mortalities.

1. Normal: Those in which no sign of mortality could be noticed.
2. Slightly affected: Those in which the effect was noticable but which could move about or made some progress while knocking the beaker.
3. Badly affected: Those which could not leave the place, showed vigour when beaker was knocked.
4. Dead: Those which showed no sign of life. Badly affected were counted among the deads for the calculation of mortality percentage.

3.6 Statistical analysis.

The statistical treatment of quantal assay data has been much aided by the development of probit analysis. This method has been widely adopted on the standard method of reducing the data to simple terms. The probit transformation is a convenient way for representing the sigmoid by a straight line.

The probit of the proportion 'P' is defined as the abscissa which corrosponds to a probability 'p' in a normal

distribution with mean 5 and variance 1; in symbols the probit of P is Y, where (42),

$$P = \frac{1}{\sqrt{(2\pi)}} \int_{-\infty}^{Y-5} e^{-\frac{1}{2}u^2} \, du \quad \ldots \quad (1)$$

when a simple normalizing transformation for the doses is available, so that x, the normalizing measure of dosage, has a normally distributed tolerance, the expected proportion of insects killed by dose x_o is,

$$P = \frac{1}{\sqrt{(2\pi)}} \int_{-\infty}^{xo} e^{-\frac{1}{2\sigma^2}(x-\mu)^2} \, dx \quad \ldots \quad (2)$$

where u and σ are the mean and variance of x.

Transforming the above equation,

$$\text{Let} \qquad Z = \frac{x-\mu}{\sigma}$$

$$dx = dz$$

$$P = \frac{1}{\sqrt{(2\pi)}} \int_{-\infty}^{\frac{x_o-\mu}{\sigma}} e^{-\frac{z^2}{2}} \, dz \quad \ldots\ldots \quad (3)$$

comparision of equations 1 and 3, show that the probit of the expected proportion killed is related to the dose by the linear equation,

$$Y = 5 + \frac{1}{\sigma}(x-\mu) \quad \ldots\ldots \quad (4)$$

Provisional regression line: From the proportions killed,

the corrosponding values of probits are obtained from the tables (43). These are known as the emperical probits. Then emperical probits and log of concentrations are plotted on ordinate and abscissa respectively. The line, best fitting through these points is called the regression line, and the corrosping probits for the same concentrations are the expected probits. Fig. no. 3 to 24 show these provisional regression lines.

Working probits: Provisional regression line drawn using the emperical probits is used to determine the weights 'w' to be attached to each observation and thus the weighted probits obtained are the working probits. The weighted regression equation of probit mortality on dosage is then computed. This is simply an improvement over first approximation.

$$w = \text{weighting coefficient} = \frac{Z^2}{PQ} \qquad \ldots \qquad \ldots \qquad (5)$$

$$Z = \frac{1}{\sqrt{2\pi}} \; e^{-\frac{1}{2}(Y-5)^2} \qquad \ldots \qquad \ldots \qquad (6)$$

where Z is the ordinate to the normal distribution corrosponding to probability P, and $Q = (1-P)$.

The tables for working probits have been prepared and values can be obtained once we know expected probits.

Adjustment for natural mortality: If in a toxicity test a proportion 'C' of test subjects would die even without any poision the total death rate expected from a dose sufficient to kill a proportion P of those which would otherwise survive is,

$$P' = C + P\ (1-C) \qquad \ldots \qquad \ldots \qquad (7)$$

providing that two types of montality operate independently. From the equation it follows that if the total proportion dead is P', the proportion killed by poision alone is

$$P = (P'-C)/(1-C) \qquad \ldots \qquad \ldots \qquad (8)$$

This is commonly known on 'Abbotts Formula'. When C is know exactl but this is not the only alteration required in probit analysis. In the weighting co-efficient w, p' is involved, therefore it has to be modified as,

$$w = \frac{Z^2}{Q\ (P + \frac{C}{1-C})} \qquad \ldots \qquad \ldots \qquad (9)$$

Tables are available for the weighting coefficient taking into consideration the natural mortality(43).

Heterogenity test: X^2 test for the data is applied to test whether the observations are homogenous or hetrogenous. The chi-square value is calculated and then it is compared with value obtained from the standard tables for the degrees of freedom equal to the number of observations minus two, and a selected level of significance. If the calculated value is less than the table value then the data is homogenous otherwise hetrogenous.

$$\chi^2 = S_{yy} - S_{xy}^2/S_{xx} \qquad \ldots \qquad \ldots \qquad (10)$$

where,

$$S_{yy} = S_{nwy^2} - S_{nwyx}\ S_{nwx}/S_{nw} \qquad \ldots \qquad \ldots \qquad (11)$$

$$S_{xy} = S_{nwxy} - S_{nwy}/S_{nw} \qquad \ldots \qquad \ldots \qquad (12)$$

$$S_{xx} = S_{nwx^2} - S_{nwx}/S_{nw} \qquad \ldots \qquad \ldots \qquad (13)$$

Here S denotes summition.

n = number of test insects

w = weighting co-efficient

x = log concentration

y = working probit.

Final regression equation: For the homogenous data the regression equation can be obtained as,

$$Y = \bar{y} + b\ (x - \bar{x})$$

where

$\bar{y} = S_y/N$

N = No. of observations

$\bar{x} = S_x/N$

b = regression co-efficient = S_{xy}/S_{xx}

Lethal concentration for 50% mortality (LC_{50}): To get the concentration for 50% mortality from the regression equation, put Y = 5, the probit value for 50% kill.

$$5 = \bar{y} + b\ (x - \bar{x})$$

$$x = x + \frac{5 - \bar{y}}{b}$$

Here x gives the log value of LC_{50} concentration.

Fudicial limits: When a parameter such as the 50% lethal concentration has been estimated from the experimental data it is natural to infer, within limits, its true value. The range within which this value lies is called fudicial limits. The exact fudicial limits to LC_{50}, the dose giving a kill whose probit is equal to 5, are shown to be,

$$X_{50}+\frac{g}{1+g}(X_{50}-\bar{x}) \pm \frac{t}{b(1-g)}\sqrt{\frac{(1-g)}{S_{nw}}+\frac{(X_{50}-\bar{x})^2}{S_{xx}}}$$

where, $g = t^2/b^2 x\ S_{xx}$

't' is the value from 't' distribution for selected level of significance. But when 't' is small compared with unity the fudicial limits can be taken as anti log of,

$$X_{50} \pm t.S.E.$$

where, S.E. = standard error $= \sqrt{V(X_{50})}$

$$V(X_{50}) = \frac{1}{b^2}\left[\frac{1}{S_{nw}} \quad \frac{(X_{50}-X)^2}{S_{xx}}\right]$$

where, $V(X_{50})$ is the variance for X_{50}.

Joint-toxicity-coefficient: The joint-toxicity coefficient of the mixture is ascertained according to the statistical procedure suggested by Sun and Johnson (44). The procedure essentially consists of the following steps.

To test the nature of the joint action of insecticides A & B, LC_{50}'s of individual insecticide and their mixtures are obtained. The toxicity indices are calculated from these LC_{50}'s

against A(or B) as standard.

(1) Toxicity index of standard insecticide A = 100

(2) Toxicity index of insecticide B $= \frac{LC_{50} \text{ of A}}{LC_{50} \text{ of B}} \times 100$

(3) Actual toxicity index of mixture M $= \frac{LC_{50} \text{ of A}}{LC_{50} \text{ of M}} \times 100$

(4) Theortical toxicity index of mixture M

= Toxicity index of A x % of A in mixture + Toxicity index of B x % of B in mixture.

(5) Joint toxicity-coefficient of mixture

$$= \frac{\text{Actual Toxicity Index of M}}{\text{Theortical toxicity index of M}} \times 100$$

The results thus obtained are to be interpreted as, "A joint toxicity co-efficient of mixture around 100 indicates probability of smilar action; independent action usually should give a coefficient less than 100 but the actual toxicity index of the mixture should be higher than the toxicity index of either component. Coefficient significantly higher than 100 indicates synergism while the value less than 100 and simultaneously the actual toxicity does not exceed by the toxicity index of the strongest toxicant, indicates antagonism".

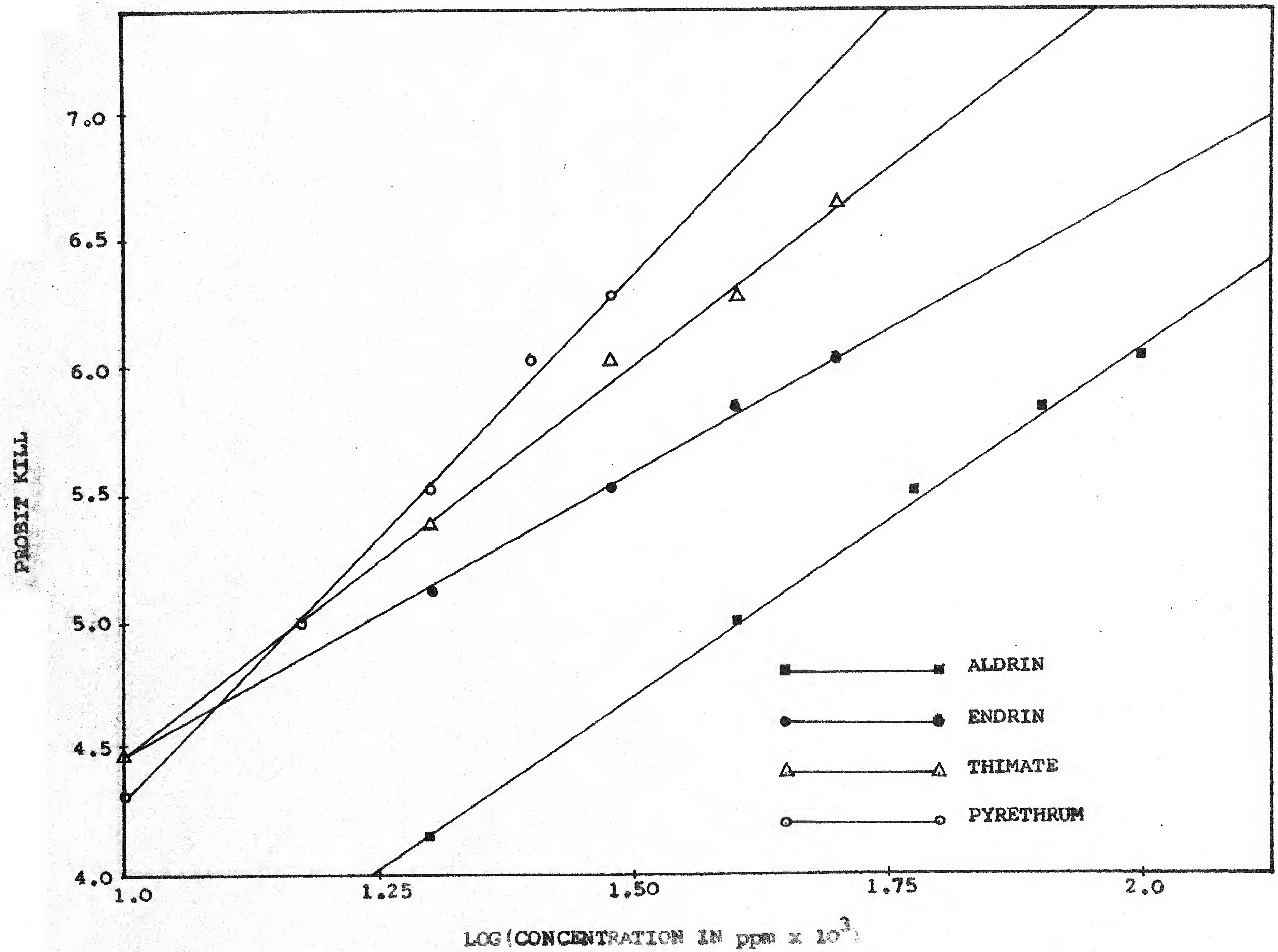


FIG. 3 PROVISIONAL REGRESSION LINE FOR ALDRIN, ENDRIN, THIMATE & PYRETHRUM

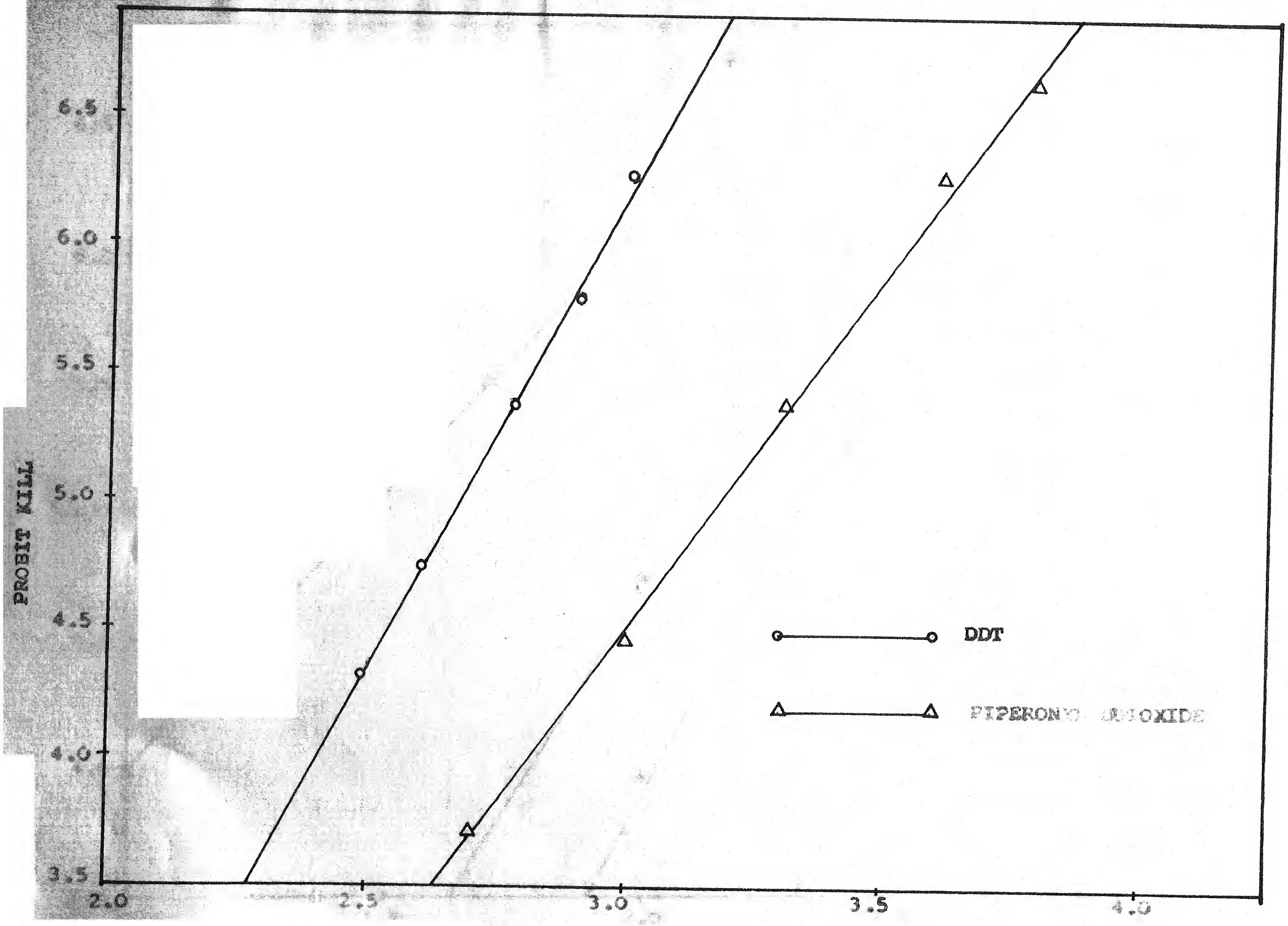


FIG. 4 PROVISIONAL REGRESSION LINE FOR DDT AND PIPERONYL BUTOXIDE

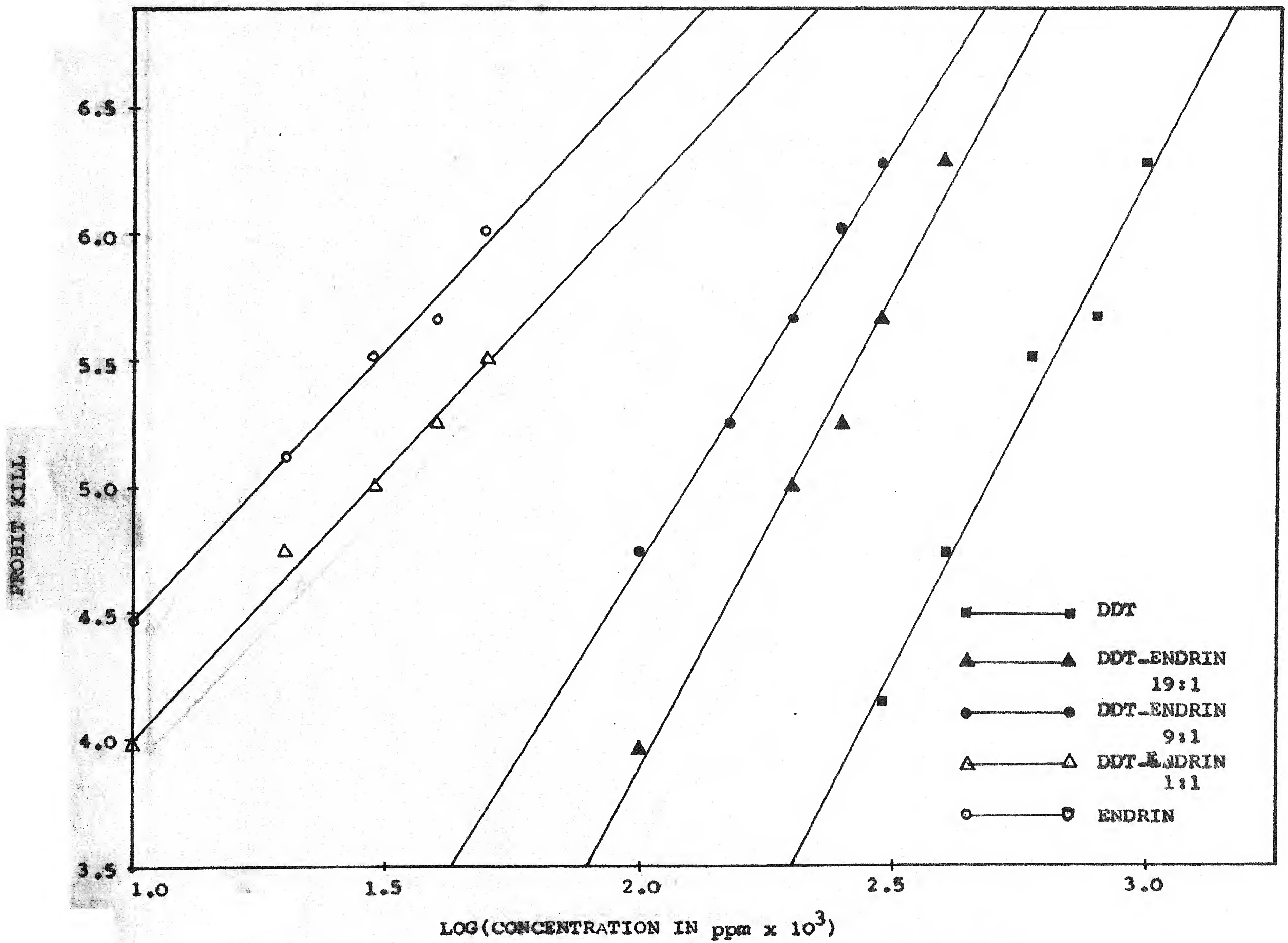


FIG. 5 PROVISIONAL REGRESSION LINE FOR DDT & ENDRIN IN PROPORTIONS, 1:1,9:1,19:1.

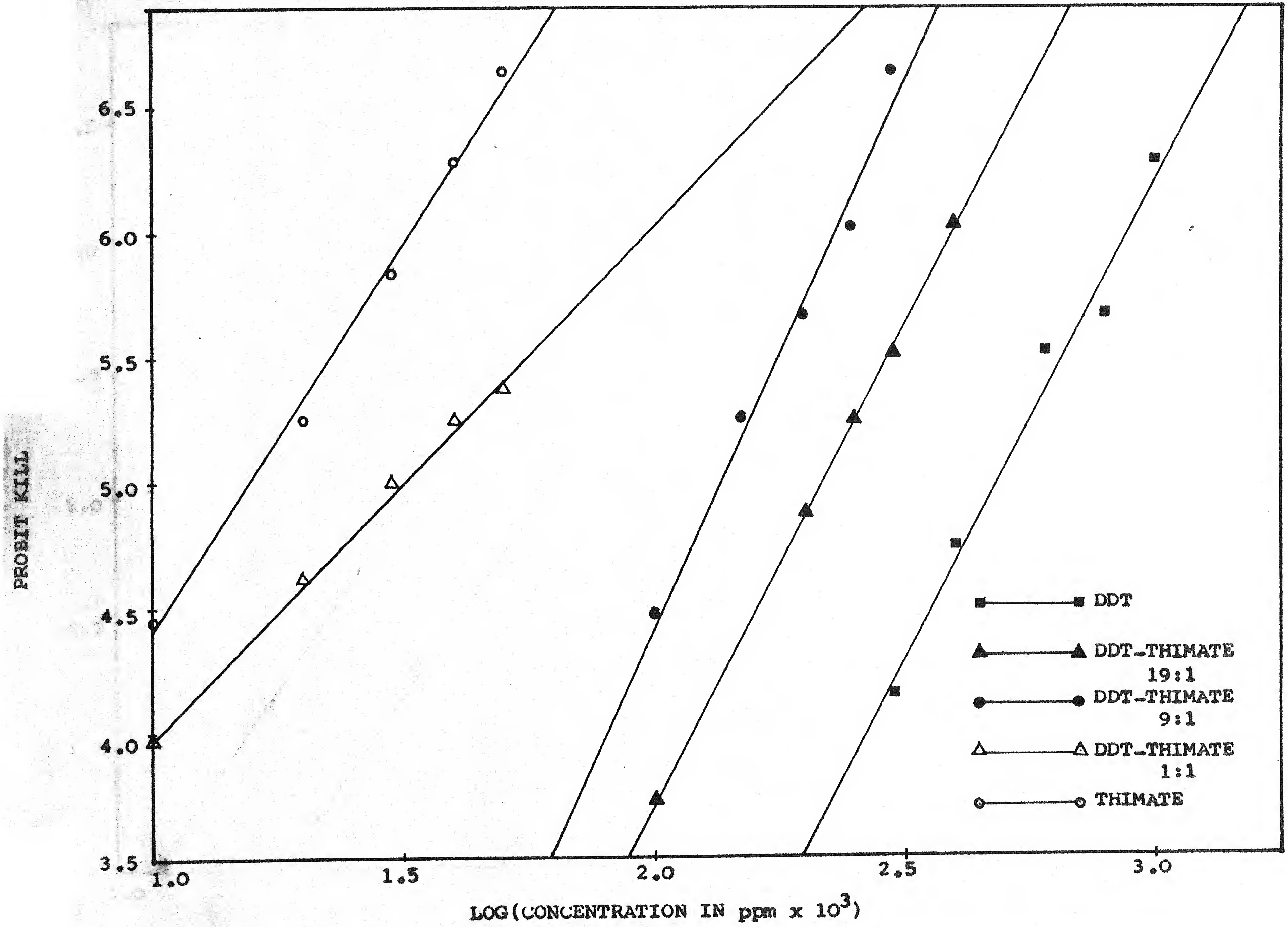


FIG. 6 PROVISIONAL REGRESSION LINE FOR DDT & THIMATE IN PROPORTIONS, 1:1, 9:1, 19:1

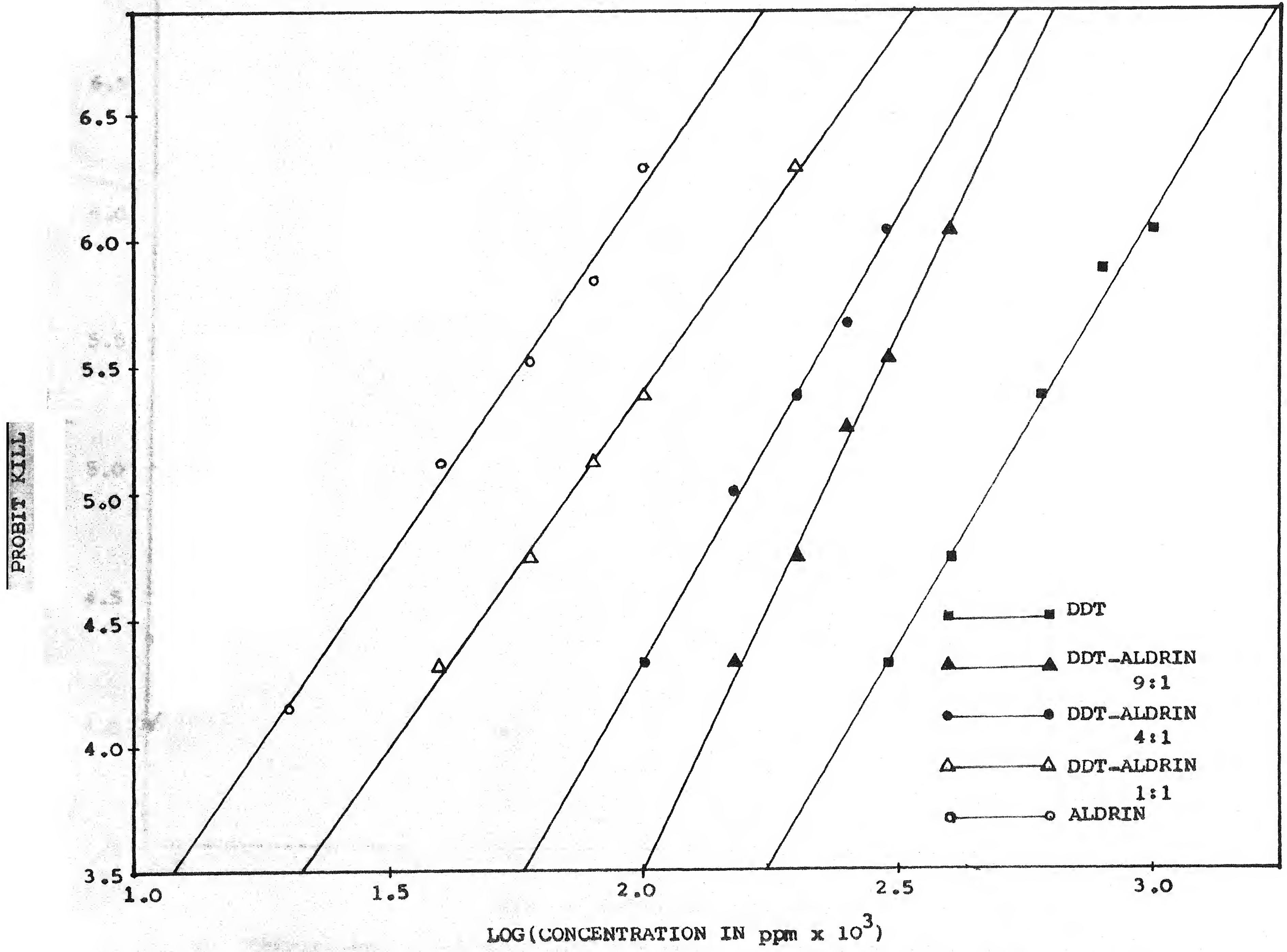


FIG. 7 PROVISIONAL REGRESSION LINE FOR DDT & ALDRIN IN PROPORTIONS, 1:1,4:1,9:1.

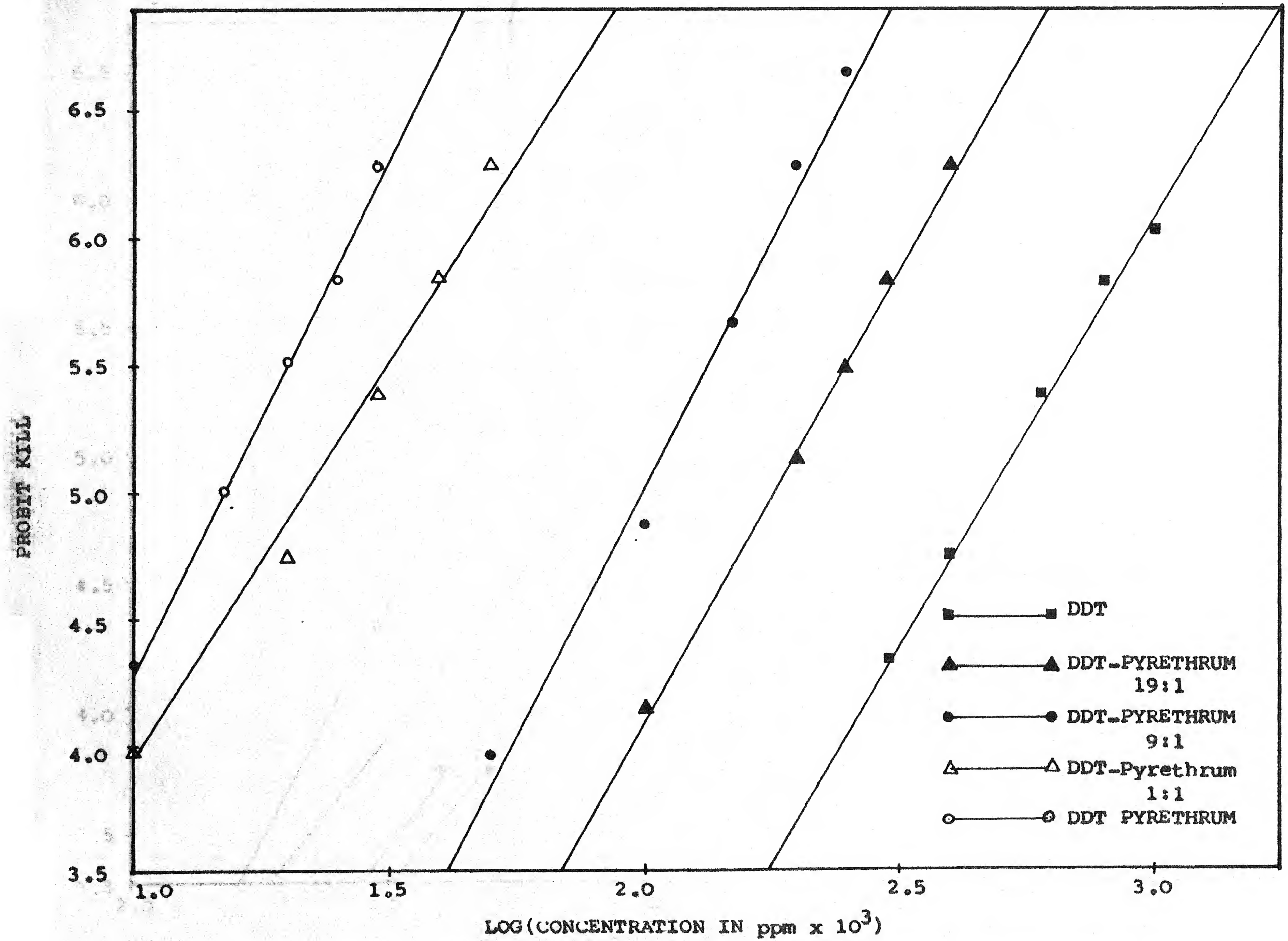


FIG. 8 PROVISIONAL REGRESSION LINE FOR DDT & PYRETHRUM IN PROPORTIONS, 1:1,9:1,19:1.

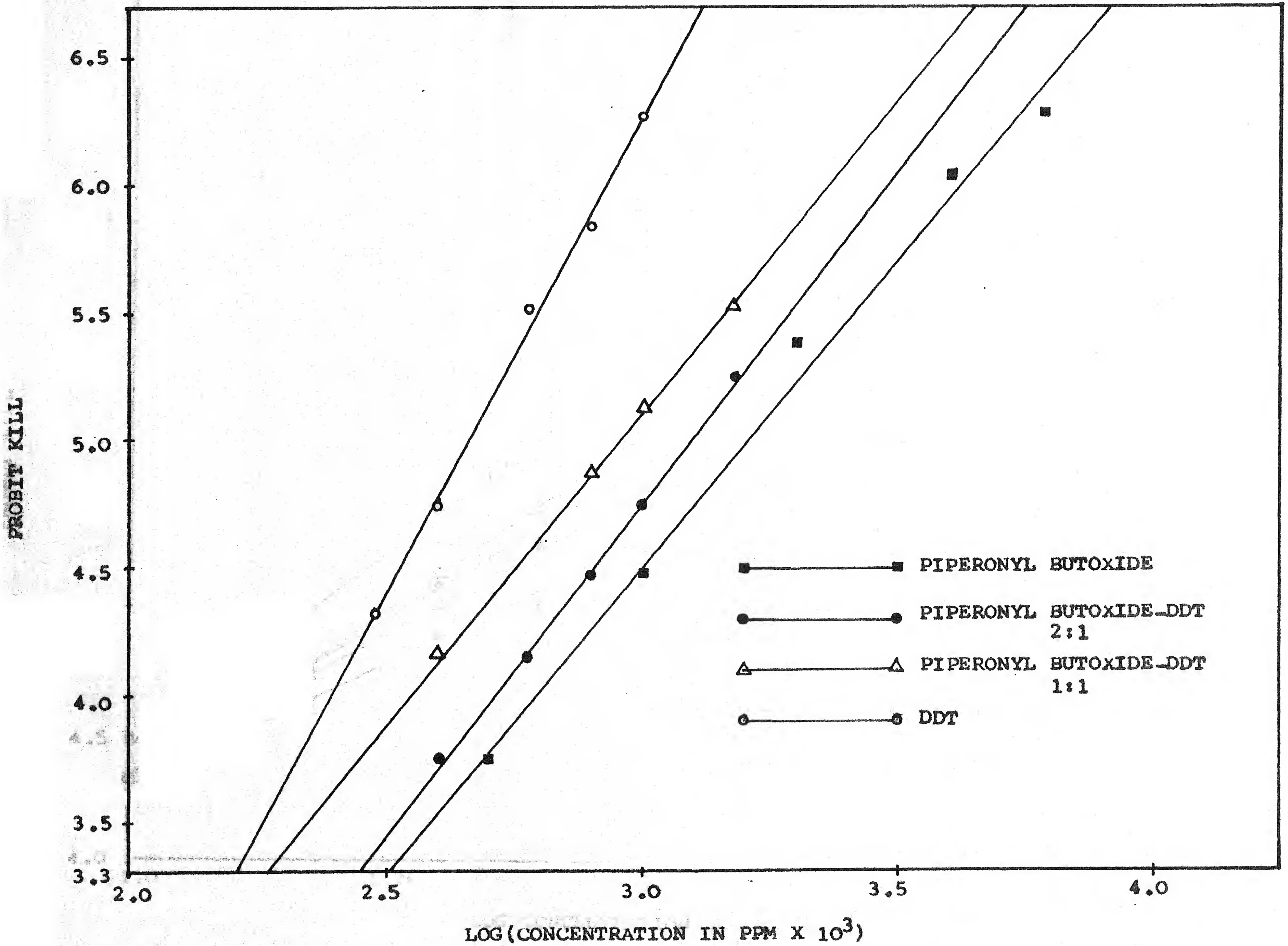


FIG. 9 PROVISIONAL REGRESSION LINE FOR PIPERONYL BUTOXIDE & DDT IN PROPORTIONS, 1:1, 2:1

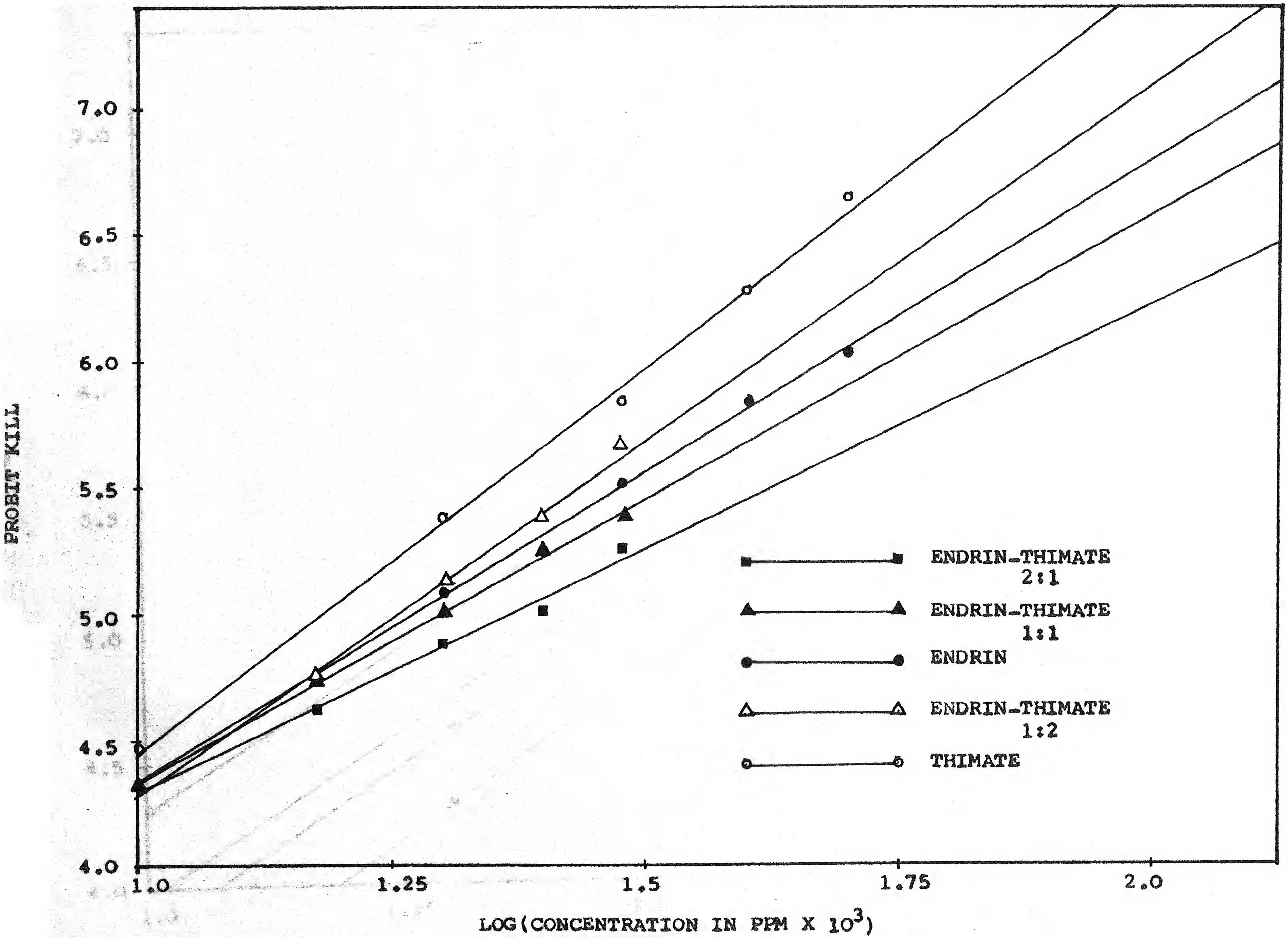


FIG. 10 PROVISIONAL REGRESSION LINE FOR ENDRIN & THIMATE IN PROPORTIONS, 1:1, 2:1, 1:2.

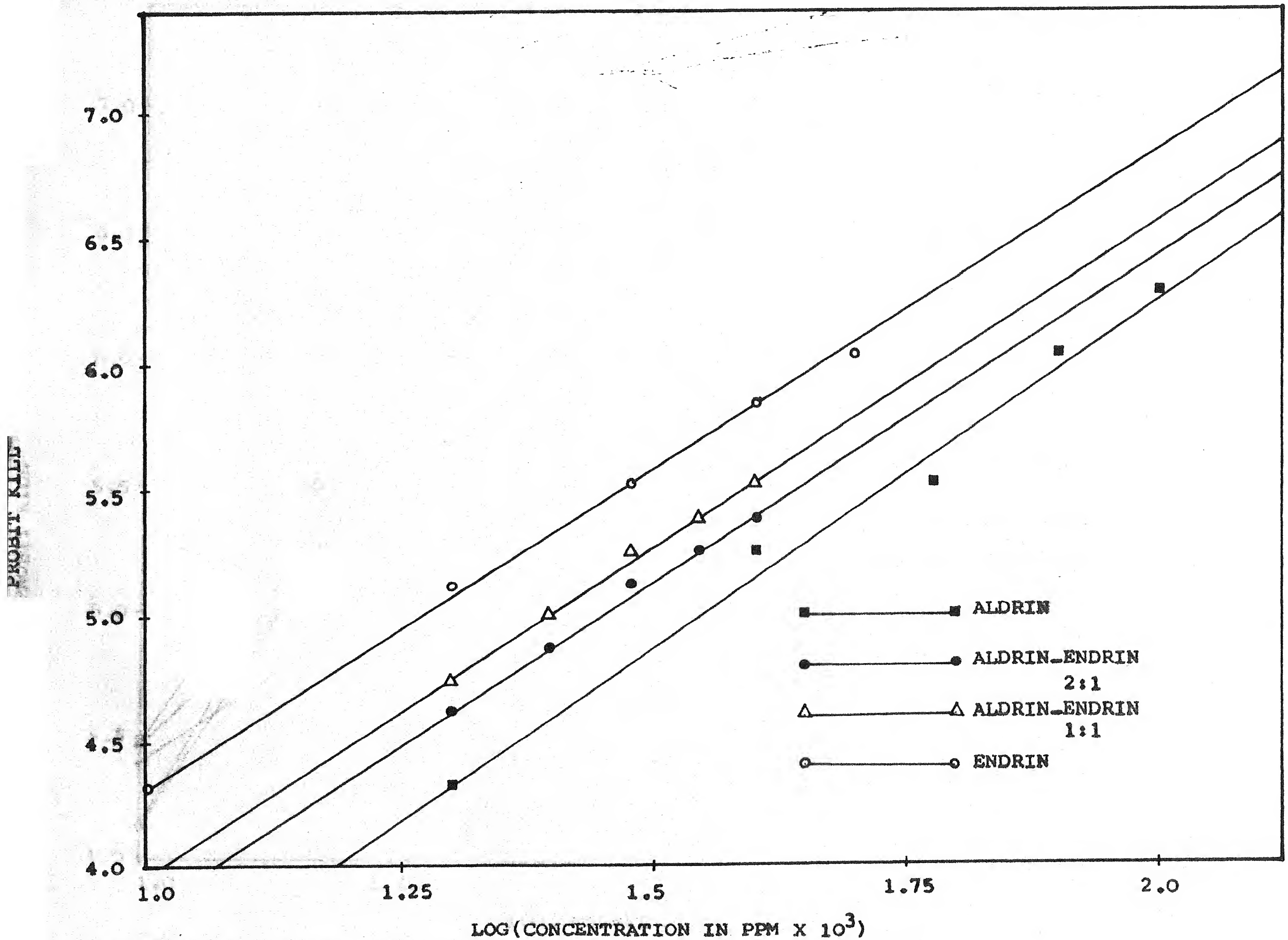


FIG. 11 PROVISIONAL REGRESSION LINE FOR ALDRIN & ENDRIN IN PROPORTIONS, 1:1,2:1.

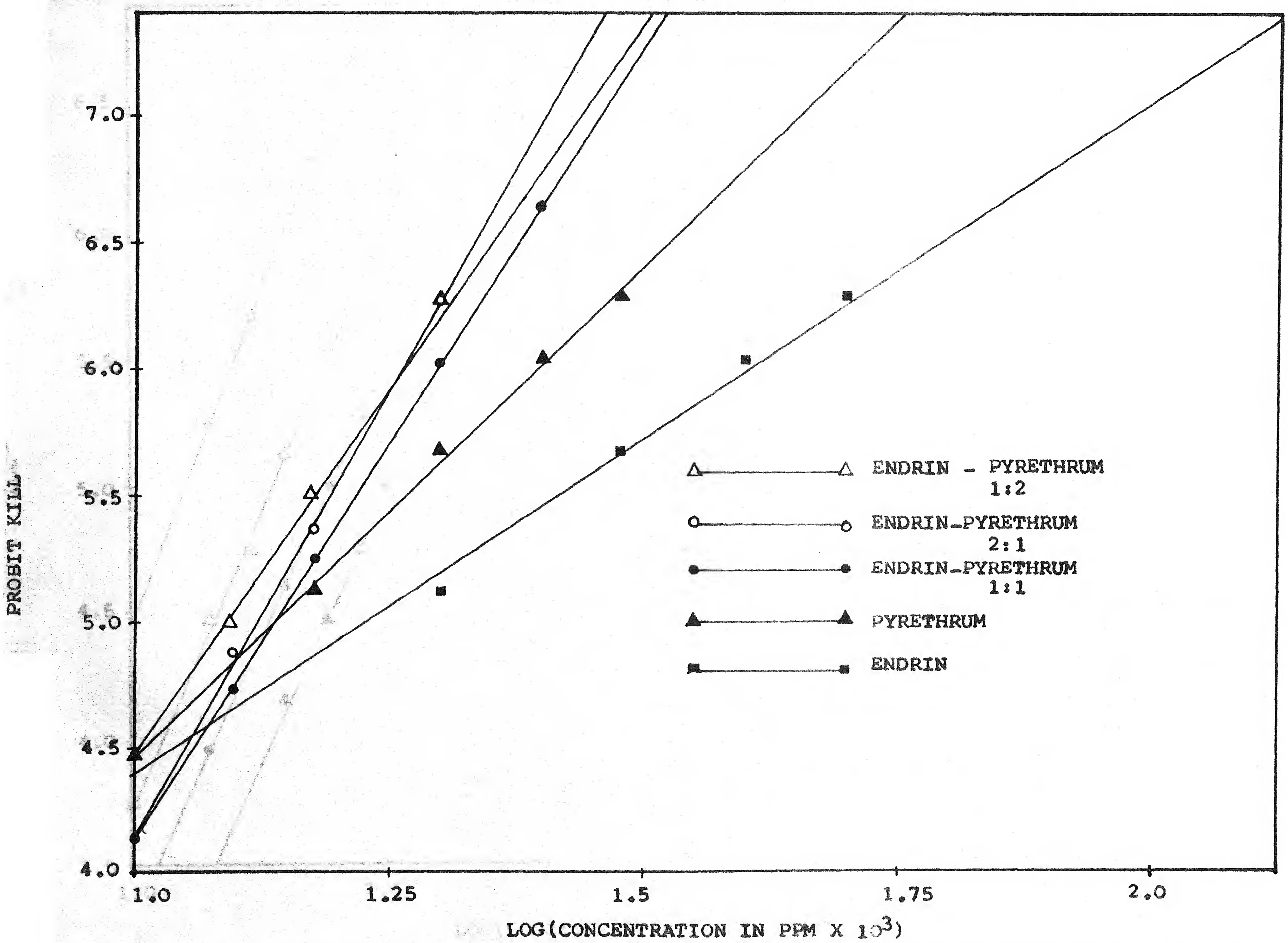


FIG. 12 PROVISIONAL REGRESSION LINES FOR ALENDRIN, PYRETHRUM IN PROPORTION, 1:1,1:2,2:1.

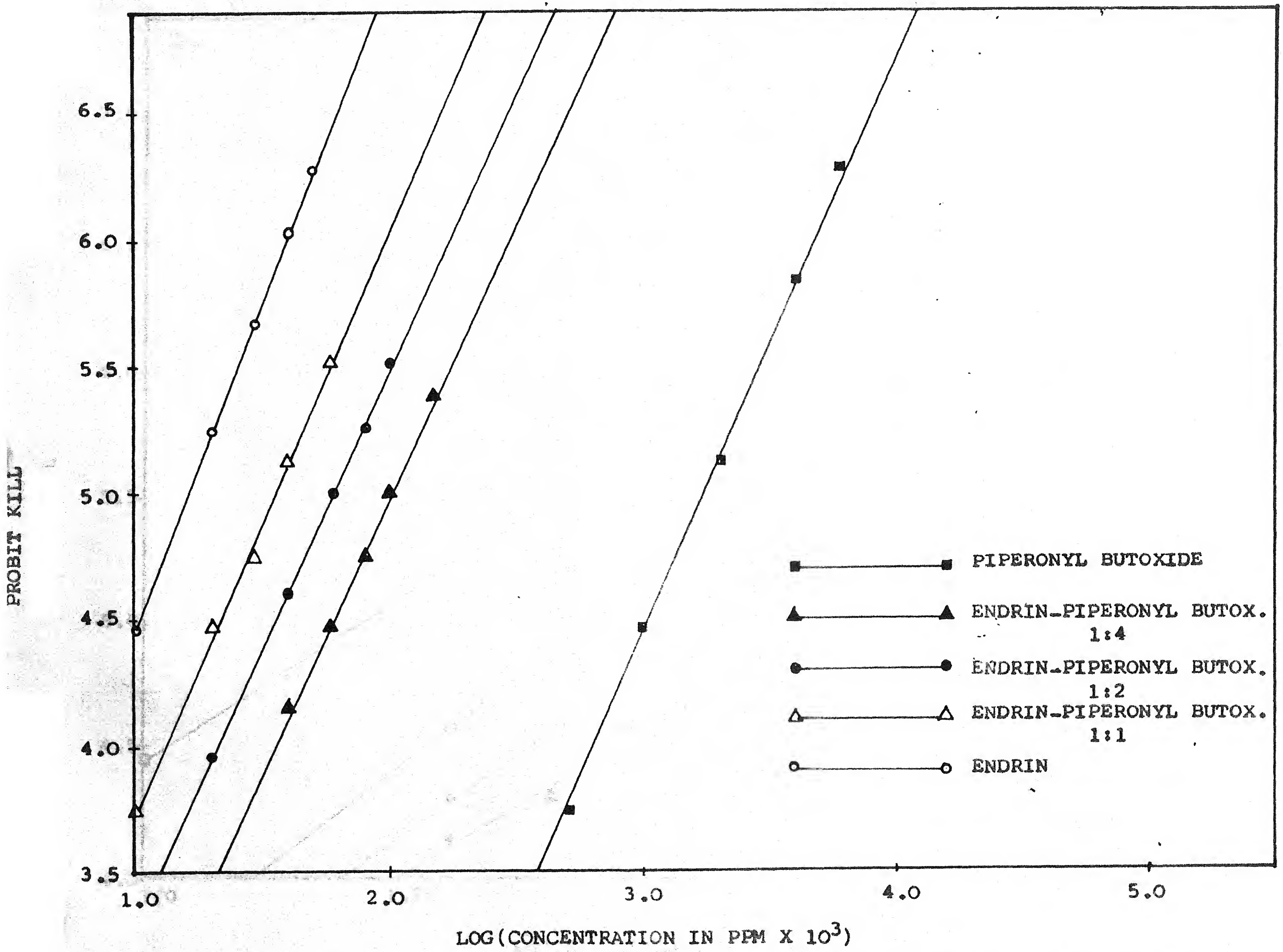


FIG. 13 PROVISIONAL REGRESSION LINE FOR ENDRIN & PIPERONYL BUTOXIDE IN PROPORTIONS 1:1, 1:2, 1:4

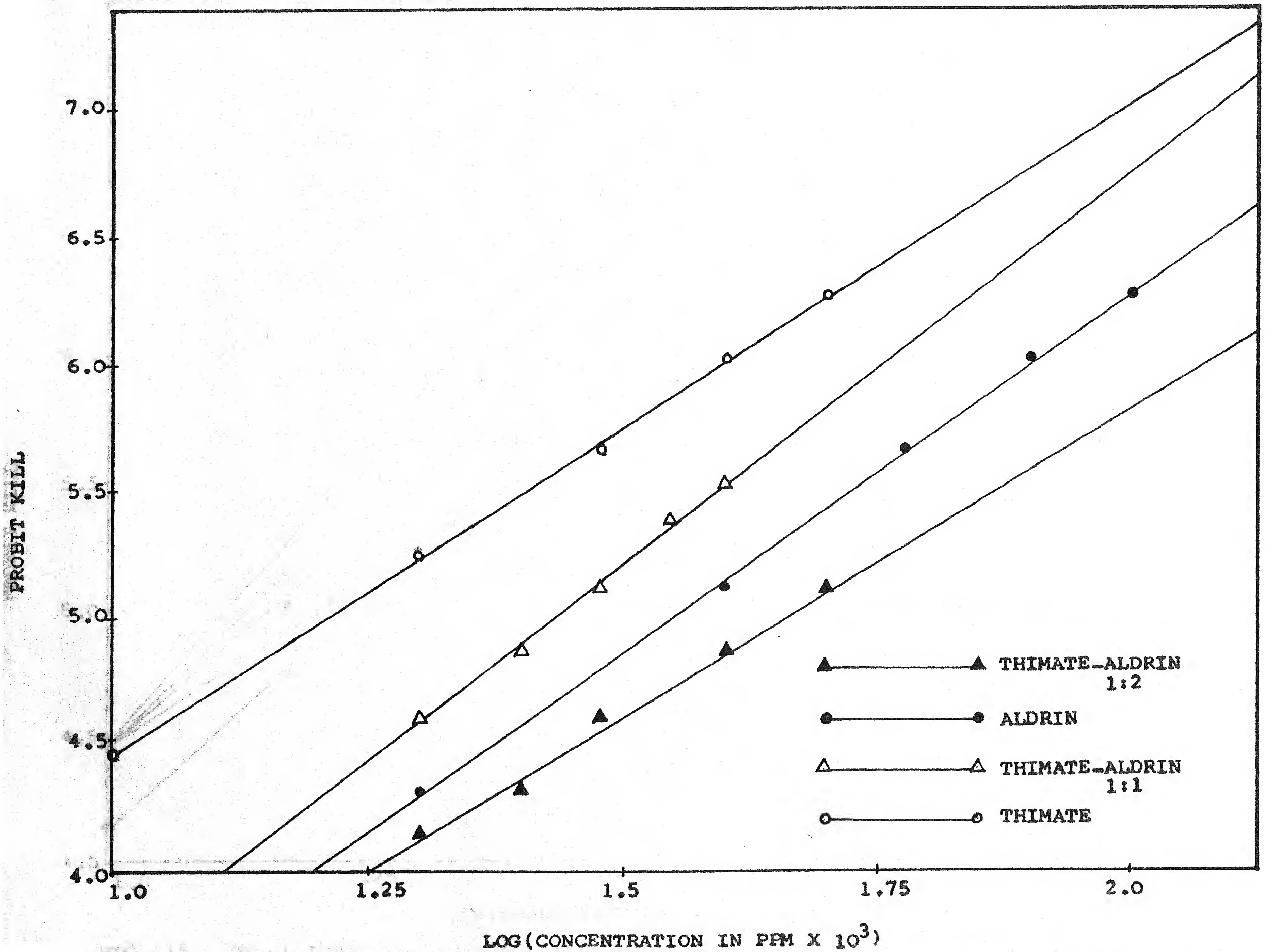


FIG. 14 PROVISIONAL REGRESSION LINES FOR THIMATE & ALDRIN IN PROPORTION, 1:1, 1:2.

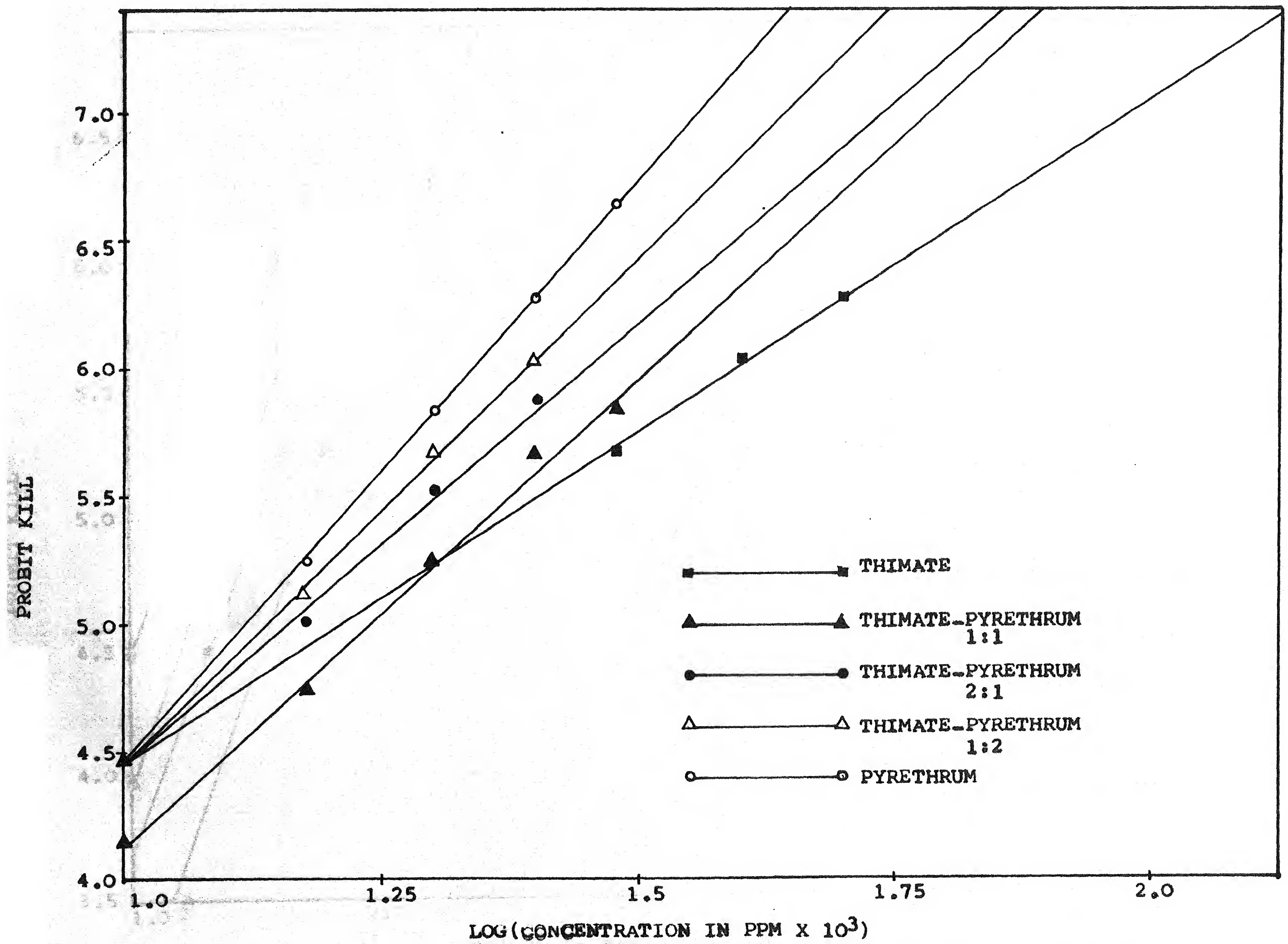


FIG. 15 PROVISIONAL REGRESSION LINES FOR THIMATE & PYRETHRUM IN PROPORTION, 1:1, 2:1, 1:2.

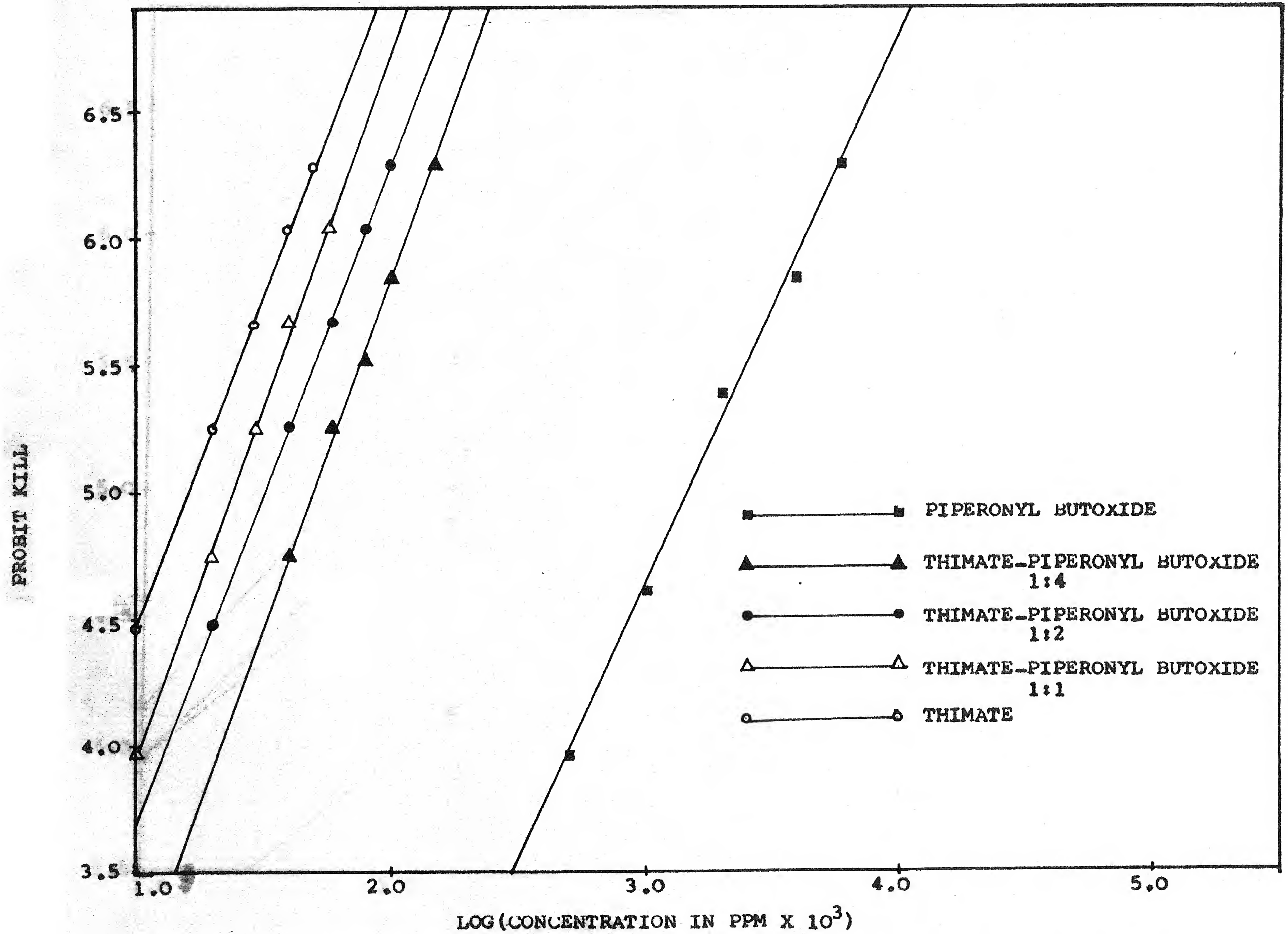


FIG. 16 PROVISIONAL REGRESSION LINES FOR THIMATE & PIPERONYL BUTOXIDE IN PROPORTION 1:1, 1:2, 1:4

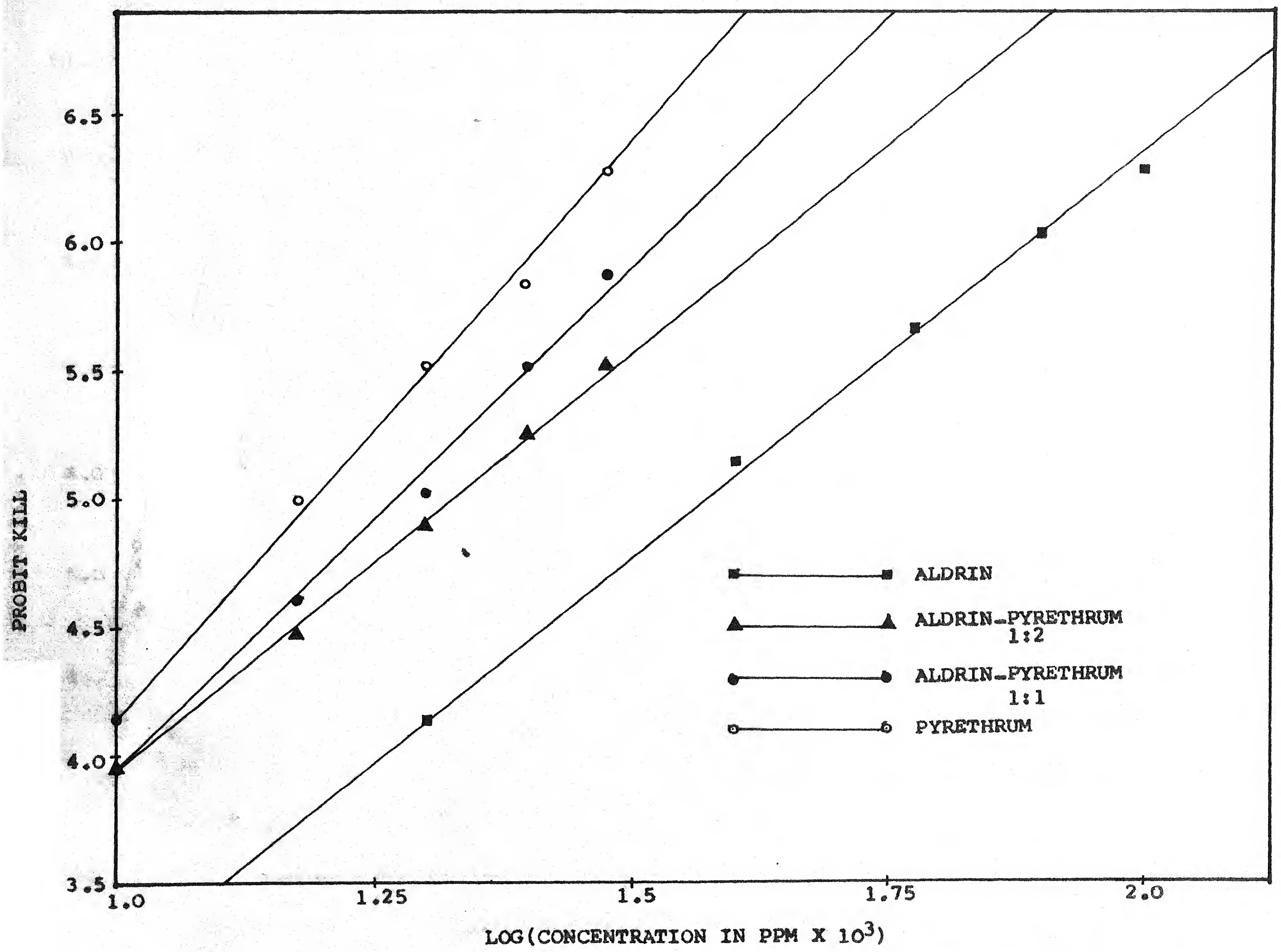


FIG. 17 PROVISIONAL REGRESSION LINES FOR ALDRIN & PYRETHRUM IN PROPORTION 1:1, 1:2.

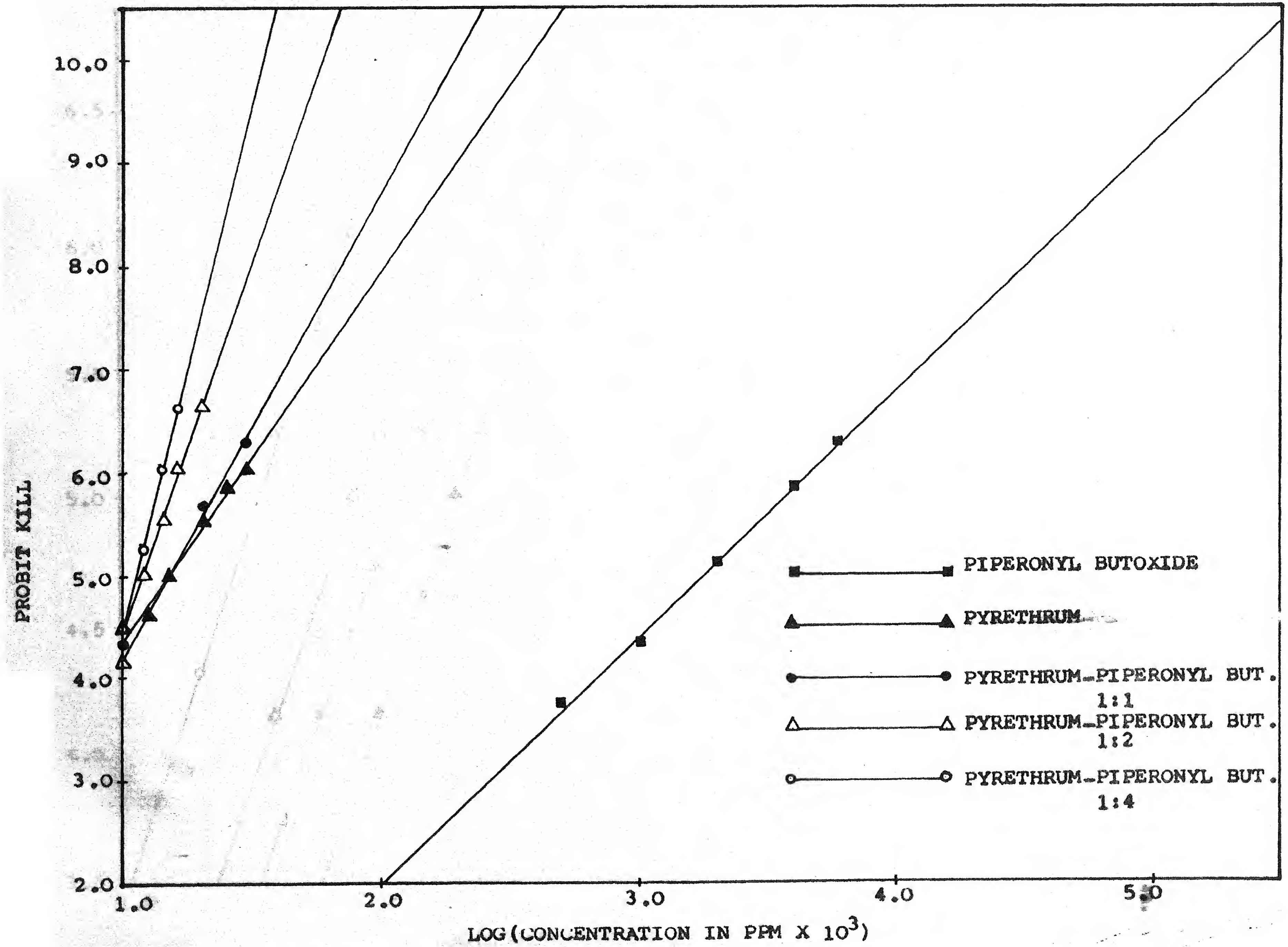


FIG. 18 PROVISIONAL REGRESSION LINES FOR PYRETHRUM & PIPERONYL BUTOXIDE IN PROPORTION 1:1, 1:2, 1:4

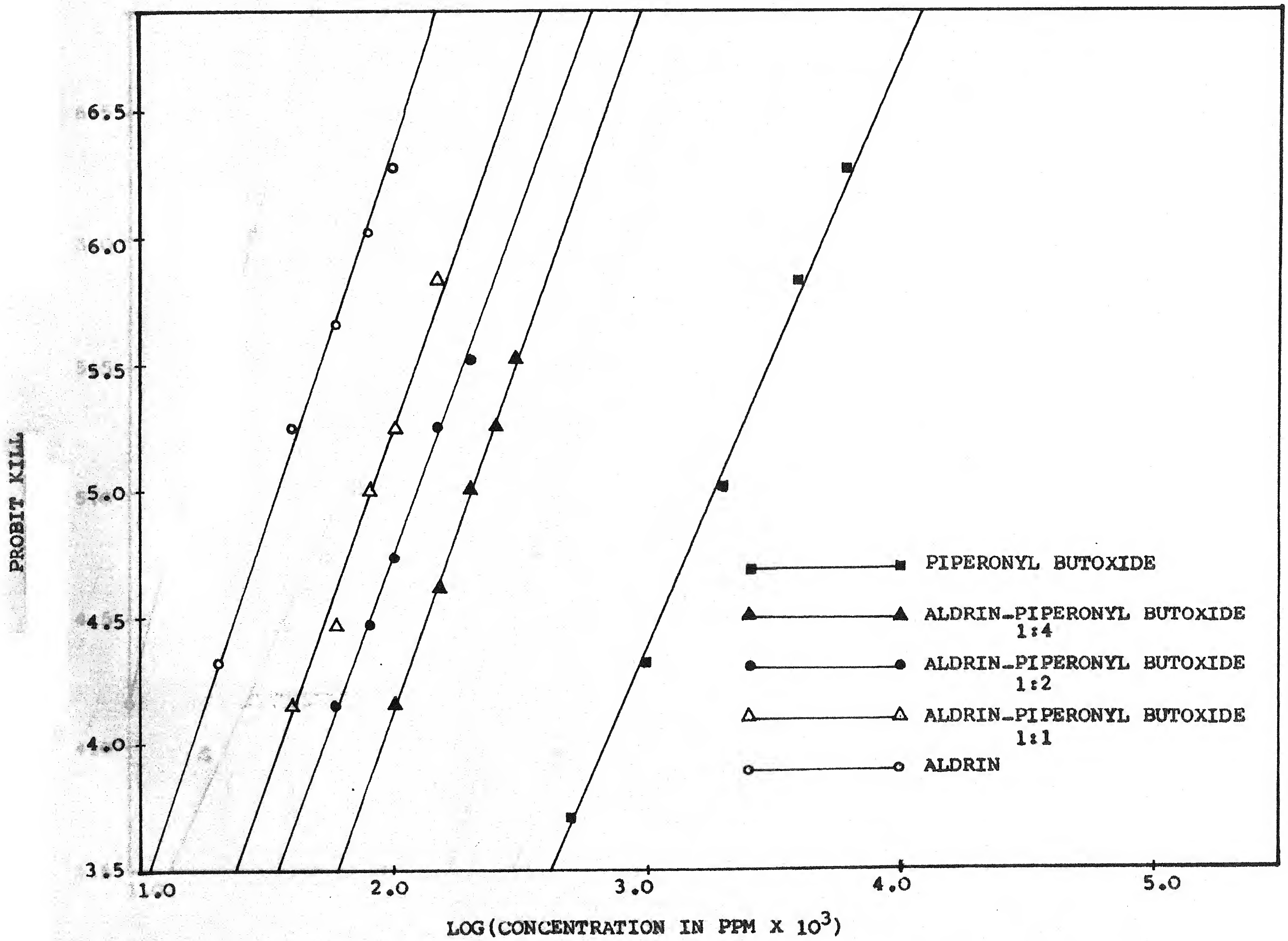


FIG. 19 PROVISIONAL REGRESSION LINES FOR ALDRIN & PIPERONYL BUTOXIDE IN PROPORTION 1:1, 1:2, 1:4

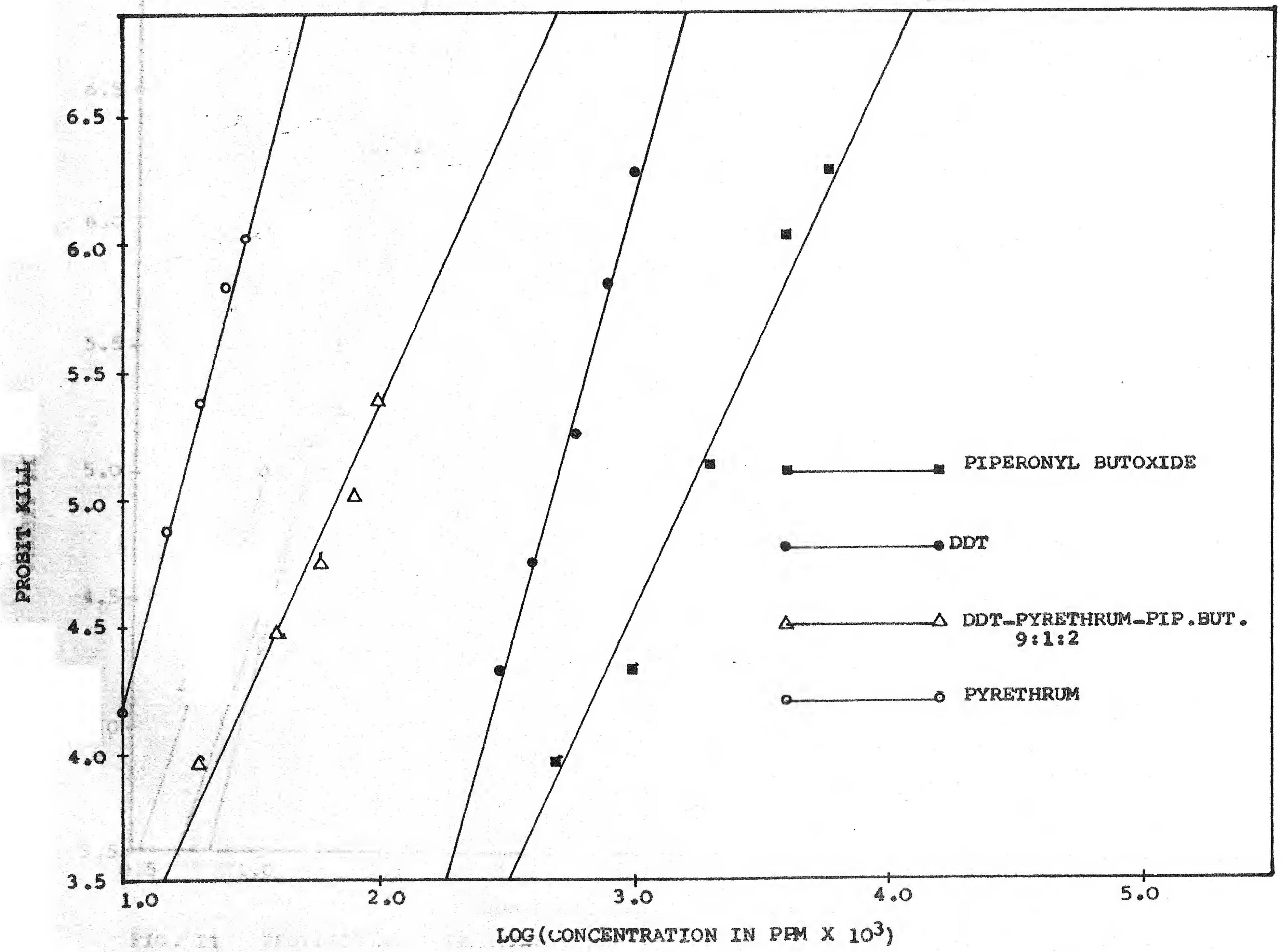


FIG. 20 PROVISIONAL REGRESSION LINES FOR DDT & PYRETHRUM & PIPERONYL BUTOXIDE IN PROPORTION, 9:1:2

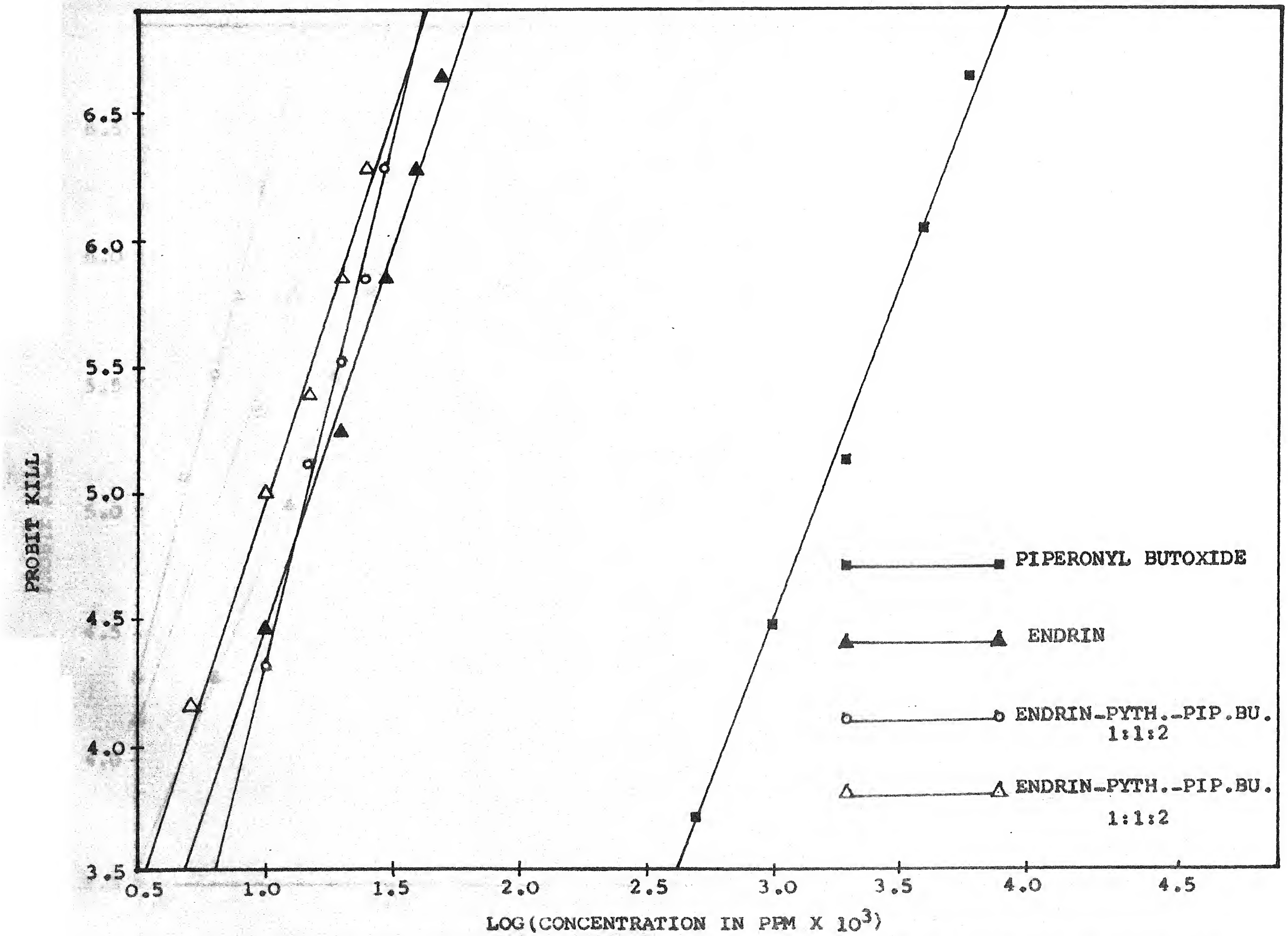


FIG. 21 PROVISIONAL REGRESSION LINES FOR ENDRIN, PYRETHRUM & PIPERONYL BUTOXIDE IN PROPORTION 1:1:2

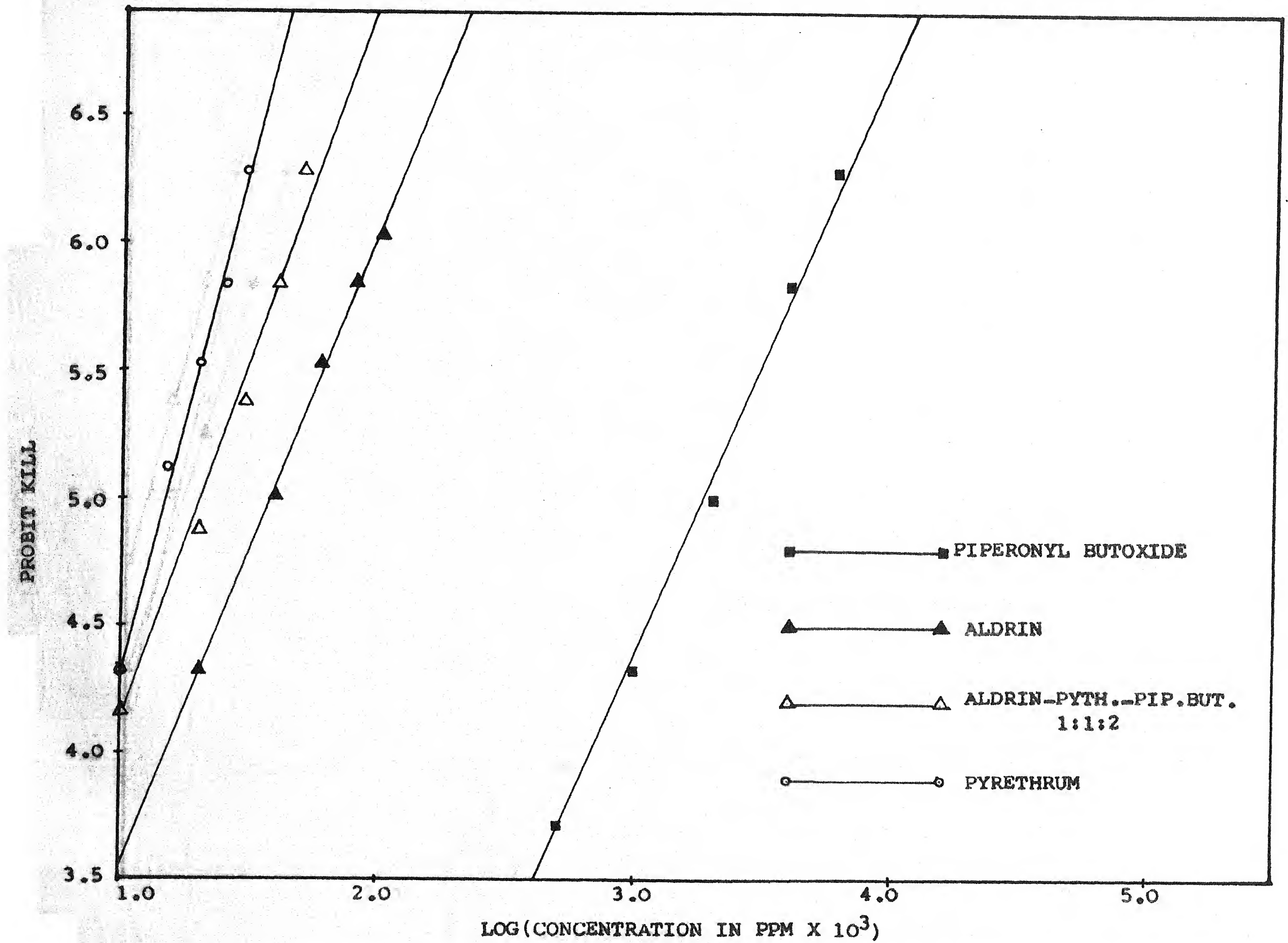


FIG. 22 PROVISIONAL REGRESSION LINES FOR ALDRIN, PYRETHRUM & PIPERONAL BUTOXIDE IN PROPORTION 1:1:2

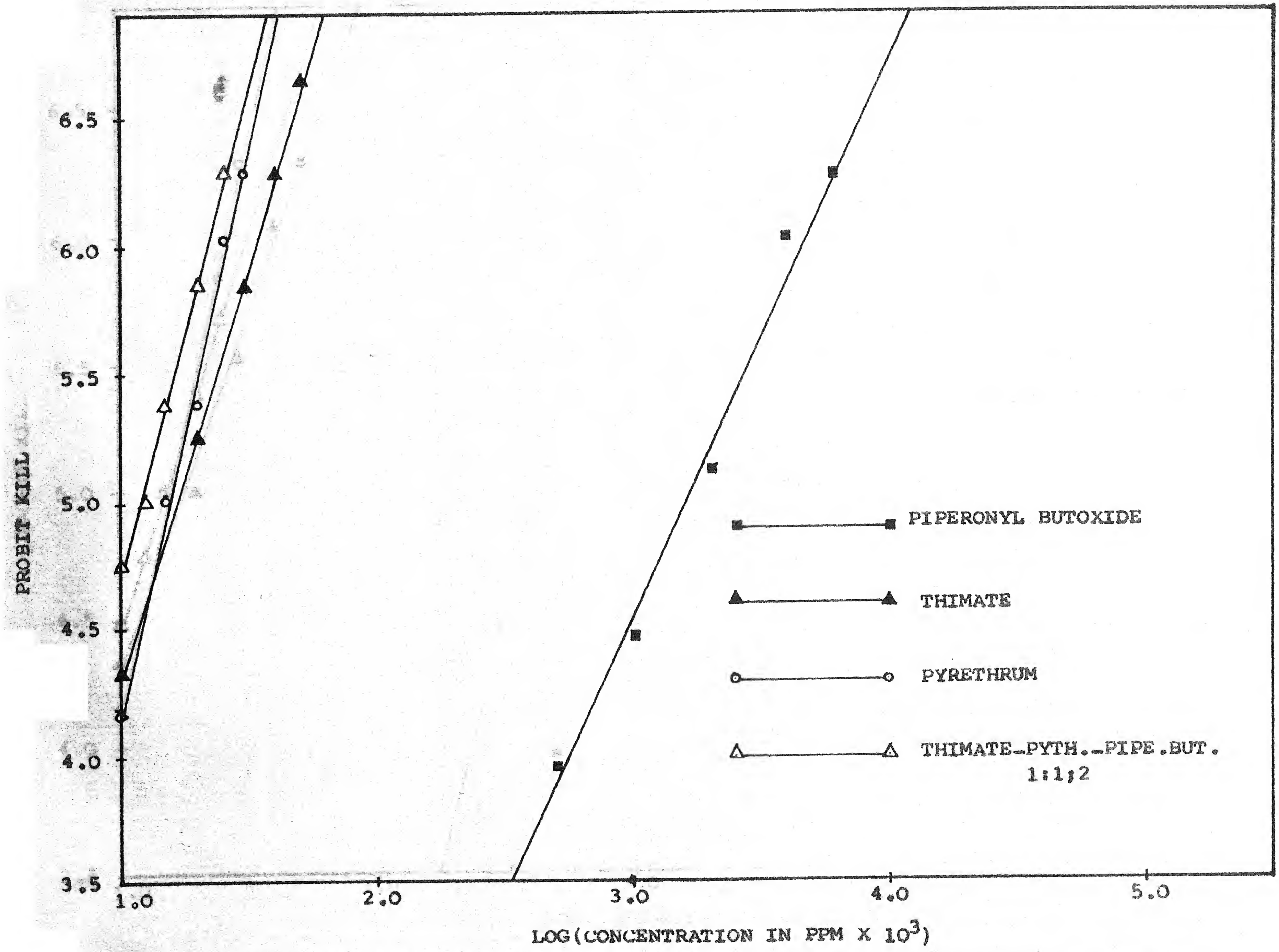


FIG. 23 PROVISIONAL REGRESSION LINE FOR THIMATE, PYRETHRUM & PIPERONYL BUTOXIDE IN PROPORTION 1:1:2

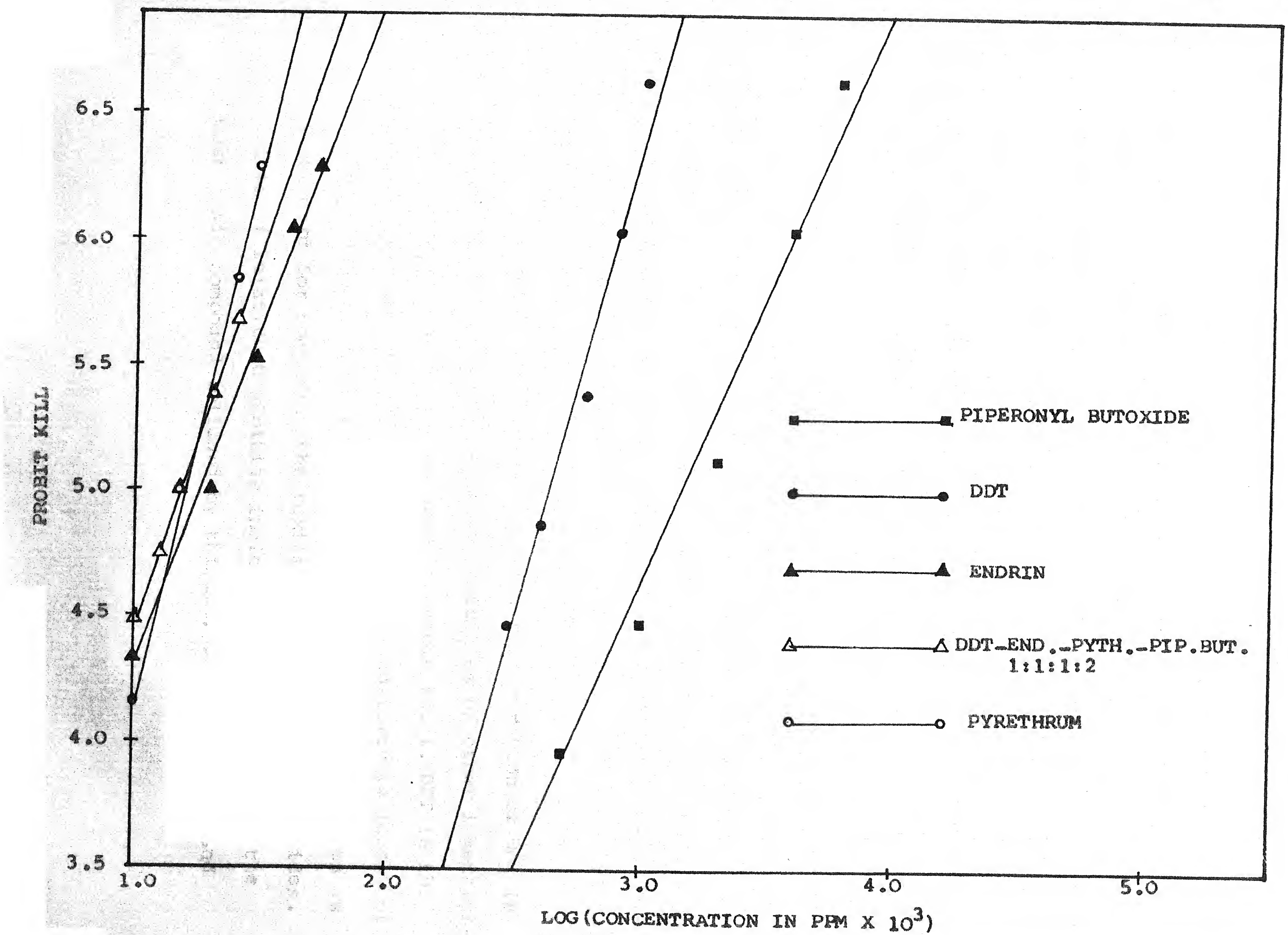


FIG. 24 PROVISIONAL REGRESSION LINE FOR DDT, ENDRIN, PYRETHRUM & PIPERONYL BUTOXIDE IN PROPORTION 1:1:1:2

4. RESULTS AND DISCUSSION

The toxicity of any biologically active chemical is normally expressed in terms of median lethal concentration i.e. LC_{50}. This concentration gives 50% mortality of test insects at a constant contact time. LC_{50} provides knowledge for comparision of relative toxicities of various insecticides. This value can also be used for evaluating the most effective combination of two or more insecticides when used jointly.

4.1 Relative toxicity of insecticides

The relative toxicity of various individual insecticides and joint toxicities of the combinations of two or more insecticides for the effective control of culex mosquito larvae will be discussed in the following pages. The values for Chi-square are calculated and compared with the table values for 5% level of significance and respective degrees of freedom. As all the calculated Chi-square values are less than table value the data is homogeneous(42).

In this work the median lethal concentration of DDT is choosen as unity and the relative toxicities of other insecticides have been calculated and shown in table no. 1. DDT is the most popular insecticide in India and its toxicity is lowest of all the insecticides used, this is why it has been choosen as a standard for comparing the toxicities of other insecticides. Values of relative toxicities indicate that pyrethrum is the most toxic compound followed by thimate, endrin, aldrin and

DDT. Piperonyl butoxide is an activator and not to be treated as an insecticide, however its relative toxicity compared to DDT is determined to obtaine information regarding its individual action.

Pyrethrum, a plant derivative attacks nervous system resulting in quick knock down of the insect. There are many reports in literature (3,11) revealing the fact that pyrethrum has been used as an effective insecticide even against DDT resistant strains. Pyrethrum has been used as a synergist also in certain cases (3,11,21,22,23).

Thimate is an organophosphorus insecticide next to pyrethrum in toxicity to culex larvae. This is 30 times more toxic than DDT. Even though specific action of thimate is not elucidated, organophosphorus compound in general are supposed to be acting upon nervous system of insects inactivating certain enzymes like acetyl-cholinesterase (41).

Among the chlorinated hydrocarbon insecticides used against mosquito larvae, endrin is most toxic followed by aldrin and DDT. It is an interesting observation that the only difference between endrin and aldrin having an oxygen replacing a double bond and the toxicity increasing two folds.

The larvae used for experimentation were collected from a villege near campus and as the DDT spraying is being done regularly in the campus, the mosquitoes might have developed

resistance to DDT. In fact comparision of the median lethal concentration determined in 1967-68 (10) for the mosquito larvae from the campus, with the present value of LC_{50}, showed that a higher dose was required which is almost six times of the previous one. This indicates that a kind of resistance is being exhibited by these larvae of the campus. In such a case the new insecticides would definitely give much promising results. However only future work with special reference to the development of resistance can give definite data about this aspect.

4.2 Action of insecticides in combination

The probit analysis data of moratality of larvae for various combinations is shown in table no. 2 while table no. 3 and 4 show the joint toxicity coefficients for these combinations. As mentioned in the literature review (19,20), there can be four kinds of actions possible from the insecticides in combinations. Action of each of the ingradient in the mixture may be similar or the action of each may be different, they may be antagonistic or synergistic to each other. The type of action can be determined by an observation of the values of joint toxicity coefficients(44). If the value is around 100, the action is said to be similar. If it is less than 100 but if the value of actual toxicity is more than the toxicity index of the most potential compound in the mixture then the action is said to be independent. If the value is more than 100 the action is synergistic while a value

less than 100 and if the value of actual toxicity of the mixture is significantly less than the toxicity index of the most potential ingradient, then it is an antagonistic action.

4.2.1 Combinations exhibiting similarity in action

Based on the above criteria the mixtures of DDT-endrin, DDT-aldrin, endrin-aldrin in different proportions seem to exhibit similar action. It seems reasonable too as all these insecticides belong to a common group i.e. chlorinated hydrocarbon and may be acting in a similar manner. Although it is not very well known about the mode of action of some of these insecticides, most of the insecticides from this group are known to act on "Sensory nerves of spontaneous discharges". The intensity of action is sufficient to cause voilent tremors (41). The tremors may last long for hours, during which period acetylcholine accumulated at the synapses inhibiting the action of esterase, that hydrolyzes acetylcholine as it forms. It was suggested that these insecticides kill the insect, exhausting its metabolic reserves through the energy expended by the tremors caused by the insecticide (4,7,41).

The action of two other combinations, namely, pyrethrum-thimate and pyrethrum-aldrin showed that the action of the components is similar. Thimate and aldrin belong to two different chemical groups while pyrethrum is a natural mixture of compounds. It is interesting to note that pyrethrum shows similar action with both thimate as well as aldrin. It is known that pyrethrum produces

paralysis in insects(41) by attacking nervous system as a whole while other insecticides act on nervous system in different ways, like acting on the sensory nerves or inhibiting cholinergic nerve transmissions. Pyrethrum seems to have similarity in action because of its wide effectivity on nervous system.

4.2.2 Combinations exhibiting independent action

Combination of organicphosphorus insecticide with chlorinated hydrocarbons indicated that the compounds are behaving independently. In all the three cases of thimate-DDT, thimate-aldrin and thimate-endrin the value of co-toxicity coefficient of the mixtures is below 100 and at the same time actual toxicity index of mixture is more than the toxicity index of most potential insecticide in the combination. Since the physiological effects produced by the different groups of compounds, independent action may be expected out of the mixture which is confirmed by these results. Evidently there may not be much of an advantage, mixing an organophosphorus compound with chlorinated hydrocarbon.

Piperonyl butoxide mixed with DDT also indicated values bordering on independent action. It can not be truely said that it is an independent action because the values of actual toxicity index of the mixture to the toxicity index of DDT are not significantly high enough to call it an independent action.

4.2.3 Combinations exhibiting antagonistic action

The values of co-efficients of toxicities of the mixture, aldrin-piperonyl butoxide and endrin-piperonyl butoxide definitely

less than 100 and the actual toxicity of the mixtures is less than toxicity index value of the most potent insecticide in the mixture. In these cases, even though the difference in toxicity indices and actual toxicity of respective mexture is not highly significant, an indication is given of the antagonistic action exhibited by them. The mode of action of piperonyl butoxide is not very clear (7). In certain cases piperonyl-butoxide showed synergistic action with an insecticide towards certain resistant strains while with the same insecticide it has shown against sensitive strain to the same insecticide, an antagonistic action(3, 11). So it is hard to conclude that piperonyl-butoxide is showing antagonistic action or independent action with sepcial reference to chlorinated hydrocarbon. Perhaps future work with more numbers of chlorinated hydrocarbon will eluciadet the role of piperonyl-butoxide.

4.2.4 Combination exhibiting synergistic action

The combination of pyrethrum with endrin and DDT showed slight synergistic action. Peculiarly pyrethrum did not show synergistic action with aldrin though it is also a chlorinated hydrocarbon. Thimate also did not show any synergistic effect with pyrethrum although piperonyl butoxide has shown synergism with thimate. Piperonyl butoxide is known to be synergistic with malathion to DDT resistant strain and antagonistic at the same time in same combination to DDT susceptible insect (3).

The mechanism of synergism is not understood till now. Infact some workers call it activation instead of synergism. Even the mode of expression of synergistic activity is completly not accepted by all workers but the present method of analysis adopted, has been widely accepted in field of insecticidal research. The best synergistic action is shown by the combination of pyrethrum and piperonyl-butoxide which is almost three times as high as the best synergistic combination discussed uptill now. Perhaps this is understandable because of the wide spectrum of actions of pyrethrum on various types of physiological systems of the insect, the attack on some of which may be activated by piperonyl butoxide (41). These interesting results promoted experiments using more than two insecticides, the insecticides that have shown some promise and tested for their joint action. These results are presented in table no. 4.

4.2.5 Combination of more than two insecticides

These experiments with more than two insecticides were of preliminary nature and require further experimentation to give basic conclusions. However some broad conclusions can be drawn from these results.

True to expectation the mixture containing aldrin gave the lowest co-efficient of toxicity. The best combination is that of endrin-pyrethrum-piperonyl butoxide. However

the mixture containing compounds DDT, endrin, pyrethrum and piperonyl butoxide has also yielded the same degree of synergism as the above. The percentage wise combination of few ingradients has less quantity of pyrethrum, a costly product, yielding the same result. Unfortunately a cost analysis could not be made because lack of information on pure components. It seems that use of proper combination of insecticides would be a best method for not only producing an effective insecticidal product but also reducing the final cost of the prepration. An other advantage in the use of combinations will be that with a lower concentration of individual, insects that may be resistant to one of the components may be knocked off. Any approach in this line has to be carefully investigated because of the danger of multiresistant strain.

TABLE NO. 1*

Results of the probit analysis of the mortality data of the insecticides used against *Culex-fatigans* larvae

Insecticide	Heterogeneity (Chi-square)**	Regression Equation	LC_{50} in ppm	Fudicial Limits	Relative toxicity
DDT	2.3697	Y=3.6607x-4.7867	0.4715	0.5087 0.4370	1.00
Endrin	2.3861	Y=2.4181x+2.0003	0.0174	0.0198 0.0153	27.50
Aldrin	2.2997	Y=2.8483x+0.5479	0.0366	0.0408 0.0328	12.88
Thimate	3.2784	Y=2.8173x+1.6441	0.0155	0.0177 0.0136	30.4
Pyrethrum	5.3513	Y=4.1110x+0.2321	0.0144	0.0156 0.0134	32.75
Piperonyl butoxide	4.6728	Y=2.3946x-2.6695	1.5953	1.7912 1.4209	0.2630

*(i) Y= probit kill
(ii) x= log (concentration x 10^3)
(iii) level of significance for heterageniety test is 5%

** Degrees of freedom for Chi-square = (n-2) where n = no. of observations. For DDT and Endrin n = 40, for Aldrin and Thimate n = 35 and for Pyrethrum and Piperonyl-butoxide n = 55.

(Computation is done by Computer IBM 7044 in FORTRAN IV LANGUAGE)
A sample calculation is shown in APPENDIX I

TABLE NO. 2*

Results of the probit analysis to the mortality data of the combinations of insecticides used against Culex-fatigous larvae

Insecticides	Proportion	Heterogeneity	Regression Equation	LC_{50} ppm	Fudicial limits
DDT & Endrin	1:1	0.1532	Y=2.1325x+1.8744	0.0292	0.0389 0.0219
DDT & Endrin	9:1	0.0346	Y=3.2421x-1.7675	0.1223	0.1636 0.0914
DDT & Endrin	19:1	0.3881	Y=3.7399x-3.5783	0.1967	0.2352 0.1645
DDT & Thimate	1:1	0.0490	Y=2.0630x+1.9188	0.0312	0.0423 0.0230
DDT & Thimate	9:1	0.2999	Y=4.1874x-3.8959	0.1332	0.1647 0.1077
DDT & Thimate	19:1	0.0097	Y=3.8180x-3.9168	0.2165	0.2555 0.1835
DDT & Aldrin	1:1	0.0413	Y=2.8110x-0.2190	0.0719	0.0900 0.0574
DDT & Aldrin	4:1	0.0709	Y=3.4594x-2.5750	0.1548	0.1886 0.1270
DDT & Aldrin	9:1	0.0897	Y=4.0863x-4.5983	0.2233	0.2607 0.1993
DDT & Pyrethrum	1:1	1.5930	Y=3.5274x+0.1912	0.0231	0.0279 0.0191
DDT & Pyrethrum	9:1	0.4634	Y=3.9175x-2.8007	0.0980	0.1200 0.0801
DDT & Pyrethrum	19:1	0.2707	Y=3.5089x-2.9123	0.1799	0.2205 0.1467
DDT & Piperonyl butoxide	1:1	0.0175	Y=2.4569x-2.2684	0.9086	1.2325 0.6699
DDT & Piperonyl butoxide	1:2	0.0196	Y=2.6948x-3.3276	1.2311	1.7319 0.8752

Endrin & Thimate	1:1	0.0192	Y=2.2408x+2.0908	0.0199	0.0259 0.0153
Endrin & Thimate	1:2	0.0596	Y=2.8111x+1.4742	0.0180	0.0222 0.0145
Endrin & Thimate	2:1	0.0717	Y=1.8932x+2.4045	0.0235	0.0337 0.0164
Endrin & Aldrin	1:1	0.0222	Y=2.6103x+1.3549	0.0249	0.0324 0.0191
Endrin & Aldrin	1:2	0.0271	Y=1.2612x+2.5842	0.0280	0.0351 0.0223
Endrin & Pyrethrum	1:1	0.0018	Y=6.2689x-2.1253	0.0137	0.0154 0.0122
Endrin & Pyrethrum	1:2	0.0215	Y=6.0493x-1.5997	0.0123	0.0140 0.0108
Endrin & Pyrethrum	2:1	0.0148	Y=7.0180x-2.8549	0.0132	0.0146 0.0119
Endrin & Piperonyl butoxide	1:1	0.1195	Y=2.2679x+1.4717	0.0360	0.0488 0.0265
Endrin & Piperonyl butoxide	1:2	0.0199	Y=2.2055x+1.0825	0.0597	0.0791 0.0451
Endrin & Piperonyl butoxide	1:4	0.0365	Y=2.1816x+0.6217	0.1016	0.1397 0.0739
Thimate & Aldrin	1:1	0.0307	Y=3.1085x+0.5473	0.0271	0.0329 0.0223
Thimate & Aldrin	1:2	0.0568	Y=2.4686x+0.9190	0.0450	0.0646 0.0313
Thimate & Pyrethrum	1:1	0.1072	Y=3.6857x+0.4470	0.0172	0.0204 0.0145
Thimate & Pyrethrum	1:2	0.0253	Y=4.3399x-0.0043	0.0142	0.0170 0.0119
Thimate & Pyrethrum	2:1	0.0167	Y=3.8754x+0.4469	0.0150	0.0180 0.0124
Thimate & Piperonyl butoxide	1:1	0.0967	Y=2.7270x+1.2266	0.0242	0.0303 0.0193

Thimate & Piperonyl butoxide	1:2	0.0075	Y=2.5795x+1.1125	0.0321	0.0446 0.0231
Thimate & Piperonyl butoxide	1:4	0.0368	Y=2.6759x+0.4649	0.0495	0.0694 0.0353
Aldrin & Pyrethrum	1:1	0.0572	Y=3.3061x+0.6133	0.0212	0.0257 0.0175
Aldrin & Pyrethrum	2:1	0.0139	Y=3.9622x-0.0244	0.0185	0.0216 0.0159
Pyrethrum & Piperonyl butoxide	1:1	0.2030	Y=4.6233x-0.4540	0.0151	0.0174 0.0132
Pyrethrum & Piperonyl butoxide	1:2	0.0697	Y=7.4192x-2.9704	0.0119	0.0133 0.0106
Pyrethrum & Piperonyl butoxide	1:4	0.0312	Y=10.6899x-6.2389	0.0113	0.0123 0.0103
Aldrin & Pipreonyl butoxide	1:1	0.2634	Y=3.0505x-0.8277	0.0814	0.0993 0.0666
Aldrin & Pipreonyl butoxide	1:2	0.0280	Y=2.6660x-0.5891	0.1249	0.1582 0.0986
Aldrin & Pipreonyl butoxide	1:4	0.0218	Y=2.8610x-1.5901	0.2011	0.2482 0.1630
DDT & Pyrethrum & Pipreonyl bu.	9:1:2	0.2629	Y=2.0797x+1.1244	0.0730	0.1032 0.0517
Endrin & Pyrethrum & Pip. but.	1:1:2	0.3367	Y=2.8769x+2.1267	0.0100	0.0128 0.0078
Aldrin & Pyrethrum & Pip. but.	1:1:2	0.3917	Y=2.9326x+1.1490	0.0206	0.0259 0.0163
Thimate & Pyrethrum & Pip.but.	1:1:2	0.1054	Y=3.8910x+0.8002	0.0120	0.0148 0.0098
DDT & Endrin & Pyrethrum &P.b.	1:1:1:2	0.0044	Y=3.0415x+1.4193	0.0150	0.0183 0.0124

*Degrees of freedom for all the combinations is 3 excepting Endrin & Pyrethrum in the proportion 1:1 and 1:2, Thimate & Pyrethrum in the proportion 1:2 and 2:1, and Pyrethrum & Piperonyl butoxide in the proportion 1:4, it is 2.

TABLE NO. 3

Co-toxicity coefficients of combinations of two insecticides tested on *Culex-fatigans* larvae

Insecticide A	Insecticide B	Proportion A:B	T.I. of insecticide A	T.I. of insecticide B	A.T. of mixture M	Th.T. of mixture M	Co-toxicity coefficient of mixture M
DDT	Endrin	1:1	100	2750	1588.0	1425.0	110.0
DDT	Endrin	9:1	100	2750	385.0	365.0	105.0
DDT	Endrin	19:1	100	2750	240.0	232.5	103.5
DDT	Thimate	1:1	100	3040	1510.0	1570.0	96.4
DDT	Thimate	9:1	100	3040	353.0	394.0	89.5
DDT	Thimate	19:1	100	3040	217.5	247.0	87.5
DDT	Aldrin	1:1	100	1288	656.0	694.0	94.8
DDT	Aldrin	4:1	100	1288	304.2	337.6	90.5
DDT	Aldrin	9:1	100	1288	211.4	218.8	97.8
DDT	Pyrethrum	1:1	100	3275	2040.0	1687.5	121.0
DDT	Pyrethrum	9:1	100	3275	481.0	417.5	115.5
DDT	Pyrethrum	19:1	100	3275	262.0	258.75	102.0
DDT	Piperonyl butoxide	1:1	100	26.30	51.9	63.3	82.0
DDT	Piperonyl butoxide	1:2	100	26.30	38.2	50.8	75.0

Endrin	Thimate	1:1	100	111.2	87.5	105.6	82.6
Endrin	Thimate	1:2	100	111.2	93.8	107.5	88.0
Endrin	Thimate	2:1	100	111.2	74.00	91.37	81.0
Endrin	Aldrin	1:1	100	47.5	69.9	74.95	93.4
Endrin	Aldrin	1:2	100	47.5	62.4	65.00	95.5
Endrin	Pyrethrum	1:1	100	120.5	127.0	110.25	115.2
Endrin	Pyrethrum	1:2	100	120.5	141.2	113.20	124.5
Endrin	Pyrethrum	2:1	100	120.5	131.9	106.80	123.5
Endrin	Piperonyl butoxide	1:1	100	10.9	48.4	74.20	65.2
Endrin	Piperonyl butoxide	1:2	100	10.9	29.2	40.58	72.0
Endrin	Piperonyl butoxide	1:4	100	10.9	17.12	28.72	59.6
Thimate	Aldrin	1:1	100	42.3	57.2	71.15	80.4
Thimate	Aldrin	1:2	100	42.3	34.25	61.50	55.7
Thimate	Pyrethrum	1:1	100	107.5	93.00	103.75	89.5
Thimate	Pyrethrum	1:2	100	107.5	109.00	105.00	103.8
Thimate	Pyrethrum	2:1	100	107.5	103.10	103.35	99.1
Thimate	Piperonyl butoxide	1:1	100	9.72	64.00	54.86	116.9
Thimate	Piperonyl butoxide	1:2	100	9.72	48.30	39.77	123.0
Thimate	Piperonyl butoxide	1:4	100	9.72	31.30	27.88	112.0

Aldrin	Pyrethrum	1:1	100	254.00	172.80	177.00	97.6
Aldrin	Pyrethrum	2:1	100	254.00	198.00	202.30	98.0
Pyrethrum	Piperonyl butoxide	1:1	100	9.05	95.50	54.50	175.0
Pyrethrum	Piperonyl butoxide	1:2	100	9.05	121.00	36.40	332.0
Pyrethrum	Piperonyl butoxide	1:4	100	9.05	127.40	37.20	342.0
Aldrin	Piperonyl butoxide	1:1	100	22.90	45.00	61.45	73.3
Aldrin	Piperonyl butoxide	1:2	100	22.90	39.30	48.30	81.4
Aldrin	Piperonyl butoxide	1:4	100	22.90	18.40	38.40	48.0

TABLE NO. 4

Co-toxicity coefficients of combinations of more than two insecticides tested on *Culex-fatigans* larvae

Insecticide A	Insecticide B	Insecticide C	Insecticide D	Proportion	T.I. of Stand. insec. A	T.I. of insecticide B	T.I. of insecticide C	T.I. of insecticide D	A.T. of mixture	Th.T. of mixture	Co-toxicity coefficient of mixture
DDT	Pyrethrum	Piper. butox.	-	9:1:2	100	3275	26.3	-	646.0	353.40	183.00
Endrin	Pyrethrum	Piper. butox.	-	1:1:2	100	120.5	10.9	-	174.0	68.2	255.00
Aldrin	Pyrethrum	Piper. butox.	-	1:1:2	100	254.5	22.9	-	177.0	100.0	177.00
Thimate	Pyrethrum	Piper. butox.	-	1:1:2	100	107.5	9.05	-	127.0	56.0	227.00
DDT	Endrin	Pyrethrum	Piper. butox.	1:1:1:2	100	2750	3275	26.3	3114	1237	254.00

CONCLUSIONS

From the studies presented in the previous chapters, the following conclusions may be drawn.

1. The studies on relative toxicities of the five insecticides showed that the toxicity against the larvae of Culex-fatigans is higher for pyrethrum, a plant product followed by thimate, an organophosphorus compound, endrin, aldrin and DDT, the chlorinated hydrocarbons.

2. The most effective combinations of two insecticides could be arranged in the following decreasing order of toxicity showing synergism.

   (i) Pyrethrum-piperonyl butoxide in the proportion, 1:4, 1:2 and 1:1.

   (ii) Endrin-pyrethrum in the proportion, 1:2,2:1 and 1:1.

   (iii) Thimate-piperonyl butoxide in the proportion, 1:2, 1:4, and 1:1.

   (iv) DDT-pyrethrum in the proportion 1:1, 9:1 and 19:1.

3. The combinations showing similar action are, DDT-endrin, DDT-aldrin, endrin-thimate, thimate-pyrethrum and aldrin-pyrethrum. As in similar action, one insecticide can be replaced at a constant proportion for the other, their mixing may not be useful unless required for a specific purpose.

4. Combinations which exhibited independent action are, DDT-thimate, DDT-piperonyl butoxide, endrin-thimate and thimate-aldrin. Their combinations may not prove to

be economical as the mortality obtained is not even equal to the sum of the two acting individually having the same concentration as in the mixture, unless any specific purpose is to be achieved.

5. Antagonistic action in the insecticide is always to be avoided and so the mixing of the following compounds should not be done as they showed antagonism. The insecticides are endrin-piperonyl butoxide and aldrin-piperonyl butoxide.

6. All the multiple combinations of insecticides showed high synergism against larvae. Piperonyl butoxide mixed with pyrethrum which are common compounds in each combination in 1:2 proporation, may be responsible for high synergism. Such combinations may be preferred for their vivid actions and may prove to be economical also. The best combination is, endrin-DDT-pyrethrum and piperonyl butoxide followed by endrin-pyrethrum and piperonyl butoxide, thimate-pyrethrum-piperonyl butoxide DDT-pyrethrum-piperonyl butoxide and aldrin-pyrethrum-piperonyl butoxide.

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APPENDIX I

COMPUTATIONS FOR THE FITTING OF A PROBIT REGRESSION EQUATION

ENDRIN-THIMATE 1:2

X	R	EMP	Y	Y_1	NW	NWX	NWY	NWX^2	NWY^2	NWXY
1.000	5.00	4.32	4.2863	4.32	10.60	10.600	45.79	10.60	197.82	45.79
1.176	8.00	4.74	4.7804	4.74	12.40	14.582	58.77	17.14	278.59	69.12
1.301	11.00	5.12	5.1313	5.12	12.60	16.392	64.51	21.32	330.30	83.93
1.398	13.00	5.38	5.4037	5.38	12.00	16.770	64.56	23.45	347.33	90.25
1.478	15.00	5.67	5.6283	5.67	11.00	16.258	62.37	24.02	353.63	92.18

$\overline{X} = 1.2706$, $\overline{Y}_1 = 5.0460$

$S_{xx} = 96.55 - \frac{74.60}{58.6}$, $S_{yy} = 1507.69 - \frac{300.01}{58.6}$, $S_{xy} = 381.28 - \frac{74.60 \times 300.01}{58.6}$

$= 1.566$ $\qquad = 12.437$ $\qquad = 4.403$

$\text{Chi-square}_{(3)} = 12.437 - \frac{(4.403)^2}{1.566}$, Regression co-efficient, $b = \frac{4.403}{1.566}$

$= 0.0596$ $\qquad = 2.811$

Contd.......

Regression equation is, $Y = 2.811x+1.4746$

Put, $Y = 5$

Therefore, $X_{50} = 1.254$

Hence, $LC_{50} = 18.0/10^3$

$= 0.018$

Varience of X_{50},

$$V(X_{50}) = \frac{1}{(2.81)^2}\left[\frac{1}{58.6} + \frac{(1.255-1.27)^2}{1.56}\right]$$

$= 0.0022$

Therefore,

$S.E. = (0.0022)^{\frac{1}{2}}$

$= 0.047$

Therefore fudicial limits for X_{50},

$= X_{50} \pm t.S.E.$

Where value of $t = 1.96$

Therefore, $= 1.254 \pm 0.092$

$= 1.346$ and 1.162

Hence Fudicial limits for LC_{50} are 0.022 to 0.0145